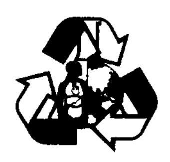
Pesticide Toxicology and International Regulation.
Edited by Timothy C. Marrs and Bryan Ballantyne

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Pesticide Toxicology and International Regulation

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Pesticide Toxicology and International Regulation

Edited by

Timothy C. Marrs

Food Standards Agency, London, UK

and

Bryan Ballantyne

*Formerly, Director of Applied Toxicology,
Union Carbide Corporation, Connecticut, USA;
Adjunct Professor, Department of Pharmacology
and Toxicology, West Virginia University, USA;
Adjunct Professor of Toxicology,
University of Pittsburgh, USA*



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Contents

Part IV Residues

Part V Human Aspects

Part VI Regulation

Preface

Pesticides are used daily and internationally on a massive scale. They have conferred immense benefits to mankind by contributing significantly to improving health and nutrition, and to the economy in the form of cheaper food. This is mainly as a consequence of their use in crop protection, food preservation, and the control of insect vectors. However, this has sometimes been at a cost since improper and/or inappropriate usage has led to small- and large-scale poisoning incidents in humans, domestic animals, and wildlife, and resulted in significant adverse phytotoxic, ecotoxic, and general environmental adverse effects. Pesticides fall into numerous chemical classes, which have widely differing biological activities and thus differing potential to produce adverse effects in living organisms, including humans. These considerations, coupled with the fact that, in addition to their use by highly trained agricultural and horticultural professionals, they are also generally available for use by less well trained or even untrained individuals, stresses the need for the control (regulation) of their release, use, and sale. This is further emphasized by the fact the pesticide industry is large, lucrative, and highly competitive. Regulation of availability, control on use and sale, and restrictions on use is carried out by competent national government (federal) authorities through their own individual pesticides safety precautions schemes, and often with due regard given to advice originating from credible international bodies such as the World Health Organization (WHO). In most scientifically and technically advanced communities the regulations and guidelines of the competent authorities now offer a considerable degree of, although not necessarily total, protection for the community. Whilst informed discussions between industry and government may be necessary and helpful, these editors believe that ultimate conclusions and decisions on clearance of pesticides should be a function of the relevant national competent authority and its independent advisory structure. It is thus important that government has available independent scientific advice from individuals of appropriate integrity.

There is a need for continual review of pesticides once they have been authorized for release (with varying degrees of restrictions) on to the market. This oversight function is required for, amongst other issues, the recognition of adverse effects not predictable or predicted, abuses and misuses, and other factors that may pose hazards to public health and the environment. This watchdog activity is sometimes, at least in some cases and in part, a function of follow-up schemes by the competent regulatory authorities who arrange for periodic reviews of pesticides following their clearance for use. In other cases, this function may be delegated to other

governmental departments, e.g. public health. In yet other cases this function may result from the activities of private (non-governmental) organizations supported by public contributions. In respect of the latter organizations, it is relevant to recall the comment of Mellanby (*Biologist*, vol. 21, p. 131, 1974), who emphasized the harm that can be done to the credibility of scientists by the pronouncements of others who are not scientists, but who use the jargon of science to promote their own objectives. Although many private organizations conduct good work and draw attention to some problems, a few others have interests more of a sociopolitical basis than genuinely humane reasons for their existence. For pesticides, an informed and balanced opinion on their benefits, and their relative safety-in-use is necessary for discussion about recommendations on the control of pesticides. In this respect, the competent authority should have credible professional advisers and advisory committees who have no vested interests in the economy (profits) of the pesticide industry but who have national and international respect for professional integrity.

There have been major changes in the regulation of pesticides (including biocides) in both the European Union and the United States of America. In the European Union the main change has been the harmonization of pesticide regulation under Directives 91/414 for agricultural and horticultural pesticides ('plant protection products') and 98/8 for biocides. Meanwhile in the United States, the Food Quality Protection Act (1996) demanded consideration of all pathways of pesticide exposure (aggregate risk assessment) and the consideration of exposure to multiple pesticides (cumulative risk assessment). Furthermore, in the United States there is progressive harmonization between the three countries (America, Canada and Mexico) of the North American Free Trade Area (NAFTA). The needs of aggregate and cumulative risk assessment has led to the questioning of current procedures for deterministic risk assessment and the consideration of probabilistic exposure assessment. So far probabilistic methodology has not been applied to the toxicology side of risk assessment, but logically it could be. Another change in pesticide regulation is the Sanitary and Phytosanitary (SPS) agreement under the Uruguay round of the General Agreement on Tariffs and Trade (GATT). The Uruguay round of GATT not only established the World Trade Organization but it was also decided that, except in certain circumstances, Codex Alimentarius Commission food standards should be used in international trade. The expert advisory committee in respect of pesticides in such circumstances is the Joint Expert Meeting on Pesticide Residues (JMPR), which is convened jointly by the World Health Organization and the Food and Agricultural Organization of the United Nations. The activity of the Organization for Economic Cooperation and Development (OECD) in developing internationally acceptable test guidelines should also not be forgotten.

Despite the moves towards harmonization, which would be expected to lead to less duplication and easier registration of active ingredients, this has not always transpired and the process has, in some ways, become more bureaucratic. Thus,

committees have proliferated like the hydra. For example, whereas there was formerly one committee in the United Kingdom dealing with pesticides, namely the Advisory Committee on Pesticides (ACP), there are now three, the ACP, the Pesticides Residues Committee (PRC), and the Biocides Consultative Committee (BCC). Also, and in the widest sense of harmonization, some countries appear to choose to ignore or apparently refuse to adopt sensible suggestions, such as harmonization of units; thus, harmonization of scientific and medical units by the United States seems to be the exception rather than the rule at both a national (federal) and a state level, although some organizations will give harmonized units in parentheses. On other scientific concepts, some agencies seem to accept without question, and without medical or scientific discussion or comment, what are to be regarded as, at the least, suspect unscientific definitions, criteria, or arguments for certain concepts. One of the most notable of these was introduced by the European Union (Council of Europe) in regard to immunologically mediated biological reactions, and notably on the definition and thus classification of substances having a sensitizing potential for the respiratory tract. The criteria for a respiratory sensitizer includes one stating (unequivocally) that for the purposes of definition and classification it does not have to be demonstrated that the material produces its sensitizing effect through an immune mechanism. This criterion was apparently the result of pseudoscientific political pressure from the representative one EEC country, and was amazingly adopted from the European Union by the OECD without question or comment. This activity, which goes contrary to current credible science, and is to be reprimanded, has several disturbing repercussions. First, it calls into question the medical and scientific credibility and membership of the appropriate EU expert committee, which flagrantly ignored internationally agreed concepts, research, and clinical findings with respiratory sensitizers. Secondly, and against widely held opinion and knowledge, the reason(s) for this pseudoscientific and unbelievable decision and action must be regarded as suspect. Finally, one practical implication is that many irritant (inflammatory-inducing) materials, without effects on the immune system, will be wrongly classified.

This book aims to bring together the regulation of pesticides with the more important aspects of their toxicology. The regulatory chapters deal with regulation in the EU, NAFTA, and Japan, respectively. Several toxicology chapters deal with insecticides, chapters being devoted to the major groups of insecticides, with one on miscellaneous insecticides. There are also chapters on fungicides, herbicides, and biocides; inevitably because of the chemically disparate nature of these compounds (particularly fungicides) compared with insecticides, these have been dealt with differently, in small groups or by individual active ingredient. Other chapters discuss biological pesticides, occupational exposure, and treatment of pesticide poisoning. It is hoped that bringing together regulation and toxicology in this way may help to stimulate more intelligent and integrated approaches to pesticide regulation and the related needs for toxicology (in all its subdisciplines) and information from other relevant disciplines. Although most regulatory authorities issue what purport

to be guidelines on data requirements for registration, the approach of some such bodies usually is not notable for flexibility, totality, and integration. It is inevitable that some companies respond accordingly.

Tim Marrs, Edenbridge, UK *July 2003*

Bryan Ballantyne, Charleston, West Virginia, USA

The views expressed do not represent those of any government department or agency.

List of Contributors

Bryan Ballantyne MD, DSc, PhD, FFOM, FACOEM, FAACT, FATS, FRCPath, FIBiol., 871 Chappell Road, Charleston, West Virginia 25304, USA

Graham M. Bell Biocides and Pesticides Assessment Unit, Health and Safety Executive, Magdalen House, Stanley Precinct, Bootle, Merseyside L20 3QZ, UK

Blaine E. Benson PharmD, DABAT, University of New Mexico College of Pharmacy, New Mexico Poison & Drug Information Center, HSCL, Rm 130, Albuquerque, NM 87131, USA

Cheryl E. A. Chaffey Pest Management Regulatory Agency, Health Canada, 250 Riverside Drive, 6606E1, Ottawa, Ontario K1A 0K9, Canada

Ian C. Dewhurst BSc, PhD, Pesticides Safety Directorate, Mallard House, Kings Pool, 3 Peasholme Green, York YO1 7PX, UK

Virginia A. Dobozy VMD, MPH, Office of Pesticide Programs, United States Environmental Protection Agency, 401 M Street SW, Washington, D.C. 20460, USA

Kannosuke Fujimori PhD, The Organization for Pharmaceutical Safety and Research and Showa University

Caroline A. Harris Exponent, 2D Hornbeam Park Oval, Harrogate, HG2 8RB, UK

Alan R. C. Hill Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK

Deborah J. Hussey BSc, Pesticides Safety Directorate, Mallard House, Kings Pool, 3 Peasholme Green, York YO1 7PX, UK

Susan L. Jordan PhD, The Dow Chemical Company, Piscataway, New Jersey, USA

Timothy C. Marrs MD, DSc, MRCP, FRCPath, FIBiol., Food Standards Agency, Aviation House, 125 Kingsway, WCZB 6NH, London, UK

Angelo Moretto Department of Environmental Medicine and Public Health, University of Padua Medical School, Padua 35127, Italy

David E. Ray MRC Applied Neuroscience Group, School of Biomedical Sciences, University of Nottingham, Queens Medical Centre, Nottingham NG7 2UH, UK

Rudy J. Richardson Toxicology Program, Department of Environmental Health Sciences, The University of Michigan, Ann Arbor, Michigan 48109, USA

Andrew Smith Medical Research Council Toxicology Laboratories, Lancaster Road, Leicester LE1 9HN, UK

Dr. Roland Solecki Federal Institute for Health, Protection of Consumers and Veterinary Medicine, Pesticides and Biocides Division, Berlin, Germany

Charles M. Thompson Department of Pharmaceutical Sciences, The University of Montana, Missoula, Montana 59812, USA

Gregory P. Wedin PharmD, DABAT, Hennepin Regional Poison Center, 701 Park Avenue, Minneapolis, MN 55415, USA

Frequently Used Abbreviations

Most abbreviations are defined in the text by the authors on their first use in individual chapters. For ease of reference, the most commonly used abbreviations are listed below in alphabetical order.

ACD	allergic contact dermatitis
ACh	acetylcholine
AChE	anticholinesterase
ACGIH	American Conference of Governmental Hygienists
ACTS	Advisory Committee on Toxic Substances (UK)
ADAC	*n*-alkyl-*n*,*n*-dimethylammonium chloride
ADI	acceptable daily intake
AEGL	acute exposure guideline level
ai	active ingredient
AMPA	aminomethylphosphonic acid
Anti-ChE	anticholinesterase
ANTU	α-naphthylthiourea
AOEL	acceptable occupational exposure level
aPAD	acute population adjusted dose
ARfD	acute reference dose
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
ATSDR	Agency for Toxic Substances and Disease Registry (USA)
AUC	area under the curve
AZA	azadirachdin
BAL	British antilewisite; dimercaprol
BChE	butyrylcholinesterase
BCF	bioconcentration factor
BEI	biological exposure index
BHC	benzene hexachloride
BNP	2-bromo-2-nitropropane-1,3-diol
BSA	bovine serum albumin
BSI	British Standards Institution
BUN	blood urea nitrogen
bw	body weight
C_{max}	peak plasma concentation

CAS	Chemical Abstracts Service
CBC	complete blood count
CDC	Centers for Disease Control and Prevention (USA)
CFIA	Canadian Food Inspection Agency
CFR	Code of Federal Regulations (USA)
CFU	colony forming unit
CHO	Chinese hamster ovary
CIREP	Cosmetic Ingredients Review Expert Panel (USA)
CMG	common mechanism group
CMIT	5-chloro-2-methyl-4-*iso*-thiazolin-3-one
CNS	central nervous system
CoA	coenzyme A
COPIND	chronic organophosphate-induced neuropsychiatric disorder
COSHH	Control of Substances Hazardous to Health (Act) (UK)
cPAD	chronic population adjusted dose
CPU	*p*-chlorophenylurea
cRfD	chronic reference dose
2,4-D	2,4-dichlorophenoxyacetic acid
DAZA	dihydroazadirachtin
DBNPA	2,2-dibromo-3-nitrilopropionamide
DDD	dichlorodiphenyldichloroethene
DDE	dichlorodiphenyldichloroethylene
DDR	2,3-de-epoxy-2,3-dihydrorhizoxin
DDT	1,1′-(2,2,2-trichloroethylidene)-*bis*-(4-chlorobenzene); dichlorodiphenyltrichloroethane
DMPS	dimercaptopropanesulfonate
DMSA	2,3-dimercaptosuccinic acid
DNA	deoxyribonucleic acid
DNOC	4,6-dinitro-*o*-cresol
DOT	Department of Transportation (USA)
DT_{50}	half-life
DT_{90}	time for 90% degradation
DTA	daily tolerable intake
DWLOC	drinking water level of concern
EBIF	ergosterol biosynthesis inhibiting fungicide
EC	European Communities
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECG	electrocardiograph
ED_{50}	dose producing (effective in producing) a 50% response
EEC	European Economic Community
EEG	electroencephalography
EINECS	European Inventory of Existing Chemical Substance
ELINCS	European List of Notified Chemicals

ELISA	enzyme-linked immunosorbent assay
EMDI	estimated maximum daily intake
EO	ethylene oxide
EPA	Environmental Protection Agency (USA)
ETU	ethylene thiourea
EUP	end use product
EUROPOEM	European predictive occupational exposure model
FAO	Food and Agricultural Organization (United Nations)
FDA	Food and Drug Administration (USA)
FEV_1	forced expiratory volume in one second
FFDCA	Federal Food, Drug and Cosmetic Act (USA)
FFP	fresh frozen plasma
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act (USA)
FQPA	Food Quality Protection Act (USA)
FS	formulated substance
FVC	forced vital capacity
GA	glutaraldehyde
GABA	γ-amino butyric acid
GAP	Good Agricultural Practice
GI	gastrointestinal
GLP	Good Laboratory Practice
GM	genetically modified
GP	general medical practitioner
GV	guideline value
h	hour
HCB	hexachlorobenzene
HCH	1,2,3,4,5,6-hexachlorocyclohexane
HGPRT	hypoxanthine-guanine-phosphoribosyl transferase
HPLC	high pressure liquid chromatography
HR	high residue level
HSE	Health and Safety Executive (UK)
I_{50}	concentration producing a 50% inhibition of enzyme activity
IARC	International Agency for Research in Carcinogenesis
IC_{50}	concentration producing a 50% inhibition of enzyme activity
IESTI	international estimate of short-term intake
IgA	immunoglobulin A
IgE	immunoglobulin E
INN	International non-proprietary name
INR	international normalized ratio
ip	intraperitoneal
IPCS	International Programme on Chemical Safety (WHO)
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry

iv	intravenous
JMPR	Joint Meeting on Pesticide Residues (FAO/WHO)
k	rate constant
K_d	ratio of sorbed to solution pesticide in water
K_{oc}	adjustment of K_d for proportion of soil organic carbon
LC_{50}	concentration causing death (lethality) in 50% of the population studied
LD_{50}	dose causing death (lethality) of 50% in the population studied
LH	luteinizing hormone
LLGL	large granular lymphocytic leukaemia
LLNA	local lymph node proliferation assay
LOAEL	lowest observable adverse effect level
LOD	limit of determination
MAFF	Ministry of Agriculture, Fisheries and Food (UK and Japan)
MBT	methylene-*bis*-thiocyanate
MCH	methylcyclohexanone
MCPA	2-methyl-4-chlorophenoxyacetic acid
ME	Ministry of Environment (Japan)
MEL	maximum exposure limit (UK)
MEST	mouse ear swelling test
MHLW	Ministry of Health, Labour and Welfare (Japan)
MIC	minimum inhibitory concentration
MIT	2-methyl-4-*iso*-thiazolin-3-one
MOE	margin of exposure
MPCA	microbial pest control agent
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRL	maximum residue limit
MRL-p	maximum residue level adjusted for change in residue concentration due to processing
MSDS	material safety data sheet
MTD	maximum tolerated dose
NAFTA	North American Free Trade Agreement
NAS	National Academy of Sciences (USA)
NCI	National Cancer Institute (USA)
NESTI	national estimate of short-term intake
NOAEL	no-observed adverse effect level
NOEC	no-observed effect concentration
NOEL	no-observed effect level
NTE	neurotoxic esterase
NTP	National Toxicology Program (USA)
OECD	Organization for Economic Cooperation and Development
OES	occupational exposure standard (UK)
OP	organophosphate

OPIDN	organophosphate-induced delayed neurotoxicity
OPIDP	organophosphate-induced delayed polyneuropathy
OPP	Office of Pesticide Programs (USA)
OSHA	Occupational Safety and Health Administration (USA)
P_{ow}	octanol-water partition coefficient
PAA	peracetic acid
2-PAM	2-pralidoxime methiodide; pyridine-2-aldoxime methiodide
PCA	*p*-chloroaniline
PCP	pentachlorophenol
PCPA	Pest Control Products Act (Canada)
PEL	permitted exposure limit (USA)
PHED	pesticide handlers exposure database
PHI	pre-harvest interval
PMRA	Pest Management Regulatory Agency (Canada)
PNEC	predicted no-effect concentration
PNS	peripheral nervous system
PoD	point of departure
P2S	pralidoxime mesylate
PSD	Pesticides Safety Directorate (UK)
PSI	peripheral sensory irritant
PT	prothrombin time
PTT	partial thromboplastin time
Quats	quarternary ammonium compounds
RAC	raw agricultural commodity
RADS	reactive airways dysfunction syndrome
RD_{50}	exposure concentration of an airborne substance causing a 50% decrease in breathing rate by nasal exposure
RfD	reference dose
SC	subcutaneous
SCB	Standard Committee on Biocides (EU)
SCE	sister chromatid exchange
SCFA	Standing Committee on the Food Chain and Animal Health (EU)
SCP	Scientific Committee on Plants (EU)
SOP	standard operating procedure
SRB	sulphate-reducing bacteria
STEL	short-term exposure limit
STMR	supervised trial medium intake
STMR-P	supervised trial medium intake for processed commodity
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
$t_{1/2}$	half-life
T3	3,5,3′-tri-iodothyronine
T4	3,5,3′,5′-tetra-iodothyronine; thyroxine
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin

TDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane
TEPP	tetraethylpyrophosphoric acid
TGAI	technical grade active ingredient
THP	*tris*(hydroxymethyl)phosphine
THPS	tetra-(hydroxyethyl)-phosphonium sulphate
TLC	thin layer chromatography
TLV	threshoid limit value
TMB-4	1,1′-trimethylene*bis*(pyridinium-4-aldoxime) dibromide
TMDI	theoretical maximum daily intake
TSH	thyroid stimulating hormone
TWA	time weighted average
UF	uncertainty factor
USDA	United States Department of Agriculture
UV	ultraviolet
v	variability factor
WATCH	Working Group on the Assessment of Toxic Chemicals (UK)
WHO	World Health Organization

Toxicity Classifications and Hazard Ratings

Several national and international systems have been developed for expressing the hazards and risks to man from exposure to pesticides and other chemicals. The main defined standards used for convenient comparative classification and cited in this book are as follows.

Toxicity classification

[1] The World Health Organization (WHO) classification for acute pesticide toxicity

Class	Hazard	Rat LD_{50} (mg/kg)			
		Peroral		Percutaneous	
		Solids	Liquids	Solids	Liquids
Ia	Extreme	≤5	≤20	≤10	≤40
Ib	High	5–50	20–200	10–100	40–400
II	Moderate	50–500	200–2000	100–1000	400–4000
III	Slight	≥501	≥2001	≥1001	≥4000

[2] The Environmental Protection Agency (EPA, USA) criteria

(*A*) *For pesticide acute toxicity*

Class	Rat acute toxicity		
	Peroral LD_{50} (mg/kg)	Percutaneous LD_{50} (mg/kg)	Inhalation LC_{50} (mg/L)
I	≤50	≤200	≤0.2
II	50–500	200–2000	0.2–2.0
III	500–5000	2000–20,000	2.0–20
IV	≥5000	≥20,000	≥20

(*B*) *For pesticide cutaneous and ocular irritancy*

Grade	Ocular effects	Cutaneous effects
I	Corrosive Irreversible corneal opacity	Corrosive
II	Corneal opacity reversible in 7 days; irritation persisting for 7 days	Severe irritation at 72 h
III	No corneal opacity; irritation reversible in 7 days	Moderate irritation at 7 h
IV	No irritation	Mild or slight irritation at 72 h

[3] European Community (EC) hazard symbols and phrases

(*A*) *Symbols*

C = Corrosive
N = Dangerous for the environment
O = Oxidizing
F = Highly flammable
F+ = Extremely flammable
T = Toxic
T+ = Very toxic
Xi = Irritant
Xn = Harmful

(*B*) *Descriptive phrases*

R9	Explosive when mixed with combustible material
R10	Flammable
R11	Highly flammable
R12	Extremely flammable
R15/29	Contact with water liberates toxic, extremely flammable gas
R20	Harmful by inhalation
R20/21	Harmful by inhalation and in contact with skin
R20/21/22	Harmful by inhalation, in contact with skin and if swallowed
R20/22	Harmful by inhalation and if swallowed
R21	Harmful in contact with skin
R21/22	Harmful in contact with skin and if swallowed
R22	Harmful if swallowed
R23	Toxic by inhalation
R23/24	Toxic by inhalation and in contact with skin
R23/24/25	Toxic by inhalation, in contact with skin and if swallowed
R23/25	Toxic by inhalation and if swallowed
R24	Toxic in contact with skin

R24/25	Toxic in contact with skin and if swallowed
R25	Toxic if swallowed
R26	Very toxic by inhalation
R26/27/28	Very toxic by inhalation, in contact with skin and if swallowed
R26/28	Very toxic by inhalation and if swallowed
R27	Very toxic in contact with skin
R27/28	Very toxic in contact with skin and if swallowed
R28	Very toxic if swallowed
R31	Contact with acid liberates toxic gas
R32	Contact with acid liberates very toxic gas
R33	Danger of cumulative effects
R34	Causes burns
R35	Causes severe burns
R36	Irritating to the eyes
R36/37	Irritating to eyes and respiratory system
R36/37/38	Irritating to eyes, respiratory system and skin
R36/38	Irritating to eyes and skin
R37	Irritating to respiratory system
R37/38	Irritating to respiratory system and skin
R38	Irritating to the skin
R40	Possible risk of irreversible effects
R41	Risk of serious damage to eyes
R43	May cause sensitization by skin contact
R44	Risk of explosion if heated under confinement
R45	May cause cancer
R48	Danger of serious damage to health by prolonged exposure
R48/20	Harmful: danger of serious damage to health by prolonged exposure through inhalation
R48/22	Harmful: danger of serious damage to health by prolonged exposure if swallowed
R48/23/24/25	Toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed
R48/23/25	Toxic: danger of serious to health by prolonged exposure through inhalation and if swallowed
R48/24/25	Toxic: danger of serious damage to health by prolonged exposure in contact with skin and if swallowed
R48/25	Toxic: danger of serious damage to health by prolonged exposure if swallowed
R50	Very toxic to aquatic organisms
R51	Toxic to aquatic organisms
R52	Harmful to aquatic organisms
R53	May cause long-term adverse effects in the aquatic environment
R59	Dangerous for the ozone layer

R61	May cause harm to the unborn child
R62	Possible risk of impaired fertility
R63	Possible risk of harm to the unborn child

Carcinogen classification

[1] International Agency for Research in Carcinogenesis (IARC) carcinogen classification groups

Group 1. The substance is carcinogenic to humans. Sufficient clinical and epidemiological evidence that the substance is carcinogenic in humans.

Group 2A. The substance is probably carcinogenic in humans. When there is limited evidence of carcinogenicity in humans but sufficient experimental evidence for carcinogenicity in laboratory animals.

Group 2B. The substance is possibly carcinogenic in humans. When there is limited evidence for carcinogenicity in humans, and less than sufficient evidence for carcinogenicity in experimental animals.

Group 3. Not classifiable with respect to carcinogenicity in humans. When there is inadequate evidence for carcinogenicity in humans, and inadequate or limited evidence with experimental animals.

Group 4. The agent is probably not carcinogenic in humans. Evidence that there is lack of carcinogenicity in humans and experimental animals.

[2] American Conference of Governmental Hygienists (ACGIH) carcinogen classification groups.

The ACGIH carcinogen categories are as follows:

A1 – Confirmed Human Carcinogen. Epidemiological evidence for carcinogenicity in humans.

A2 – Suspected Human Carcinogen. Human data are adequate, but conflicting or insufficient to confirm human carcinogen. Carcinogenic in experimental animals at doses, by routes of exposure, at sites, of histopathological type, or mechanisms relevant to worker exposure. Limited human data but sufficient animal data.

A3 – Confirmed Animal Carcinogen with Unknown Relevance to Humans. The substance is carcinogenic in experimental animals at high doses, by route(s),

at sites, of histopathological types, or by mechanism(s) that may be relevant to human exposure. Available epidemiology does not confirm an increased risk of cancer in exposed humans.

A4 – *Not Classifiable as a Human Carcinogen*. Lack of information excludes a definitive assessment of potential carcinogenicity in humans. *In vitro* or animal studies do not provide positive indications of a carcinogenic potential.

A5 – *Not Suspected as a Human Carcinogen*. Based on well conducted epidemiological studies, or when the evidence suggesting a lack of carcinogenicity in experimental animals is supported by mechanistic studies.

(Complete details of descriptions and derivations to be found in ‘‘Guidelines for the Classification of Occupational Carcinogens’’, *Documentation of the Threshold Limit Values and Biological Exposure Indices*, ACGIH, Cincinnati, Ohio.)

Pesticide Toxicology and International Regulation.
Edited by Timothy C. Marrs and Bryan Ballantyne
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1 Pesticides: An Overview of Fundamentals

Bryan Ballantyne and **Timothy C. Marrs**

Definition and introductory generalizations

The number of substances that fall into the major descriptive class of pesticides, and its various chemical and biological subgroups, is enormous. To achieve their ultimate major intended function pesticides are introduced into the environment to control by harming, usually by killing, those living organisms ('pests') that are detrimental, or potentially detrimental, to the existence or health of the human race. This broad definition of pesticides often excludes those biologically active substances that are used to control or eliminate organisms that directly infect humans (and domestic animals) and cause ill health, e.g. antibiotics to control bacterial infection. However, there are some materials which are common, and overlap is inevitable, e.g. materials used to control fungi in agricultural or horticultural situations and those used therapeutically against pathogenic fungi in human medical practice. The word (description) pesticide in most discussions is used to cover substances that control organisms (insects, fungi, plants, slugs, snails, weeds, micro-organisms, nematodes, etc.) which destroy plant life and interfere with the food chain, and which act as vectors for disease organisms to man and animals. This generic definition is frequently extended, rather unsatisfactorily and inaccurately, to cover other chemicals used on plants, such as growth regulators. This may sometimes be a reflection not of 'classification accuracy', but rather of an autocratic approach by the relevant competent authority having responsibility for control and regulation of the material(s). From a legal point of view, pesticides are defined in various ways in different countries. A simplistic dictionary definition of a pesticide might be: 'a substance that is used to kill unwanted living organisms'. However, some definitions are wide ranging and complex; for example, under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) pesticides are defined as including,

> (1) any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest [insect, rodent, nematode, fungus, weed, other forms of terrestrial or aquatic plant or animal life or viruses, bacteria, or other micro-organisms, except viruses,

> bacteria, or other micro-organisms on or in living man or other animals, which the Administrator declares to be a pest] and (2) any substance or mixture of substances intended for use as a plant regulator, defoliant or desiccant (40 CFR 162.3).

Some authorities no longer appear to legislatively refer to ‘pesticides’. For example, the European Union writes of plant protection products and biocides. ‘Plant protection products’ are defined as chemical or biological products intended to: protect plants or plant products against harmful organisms; influence the life processes of plants, other than as a nutrient (e.g. growth regulators); preserve plant products; destroy undesired plants or parts of plants; and check or prevent undesired growth of plants. Biocidal products cover a wide range of products, including: disinfectants (bacteria and viruses), preservatives (mould, fungi, and insects), public hygiene insecticides (e.g. flies, mosquitoes, ants), rodenticides (rats, mice), and antifouling preparations.

Because of their intended use to cause harm to living organisms, pesticides may also produce toxic (adverse) effects in other lower and higher organisms, including man, sometimes by a common mechanism, but in many other cases by a co-incidental and differing physical or biochemical property of the molecule to harm biological material. As discussed below, and throughout this book, these considerations have multiple implications, including the potential for small-scale and large-scale adverse effects on the environment (ecotoxic, phytotoxic), on domestic animals, and on man. This reflects itself in the considerable and wide-ranging detail requested by competent authorities in advance of discussions on approval of pesticides for use, even in limited scale trials. The need for an independent and ethical professional expert review by the relevant competent authority, and provision for appropriate accessible documentation (with reservations only for data covered as, and agreed as, competitive ‘trade secrets’) is clear. So also is a requirement for transparency of the processes on the part of both competent authorities and industry.

Major historical features

Amongst the earliest pesticides were natural products such as insecticides, nicotine and rotenone, extracted from tobacco and derris root. Copper fungicides (e.g. Bordeaux mixture, a mixture of copper sulphate and calcium hydroxide, introduced in the 1880s) were long a mainstay for protection against fungi and are still used to some extent. Other inorganic chemicals that that have been, or are, used as pesticides include calcium and lead arsenate and sulfur, and common salt and sodium chlorate have been used as herbicides. A revolution began in the 1930s. A programme investigating the insecticidal properties of organic phosphorus compounds was undertaken by the German company IG Farbenindustrie, to develop synthetic insecticides, the work being lead by Gerhard Schrader. In the latter half of the 1930s, the Hitler government required that information on toxic compounds should be reported to the War Ministry. Compounds thus reported included tetraethyl pyrophosphate (TEPP), as well as tabun

and sarin (GA and GB), the two earliest G-type chemical warfare agents (Schrader, 1963). Further development of insecticidal organophosphates (OPs) brought greater specificity in their toxicities to insects versus non-target species such as humans (Marrs, 2001). The introduction of the organochlorines came more or less at the same time as that of the OPs: hexachlorocyclohexane and, shortly afterwards, dichlorodiphenyltrichloroethane (DDT) were discovered in the 1940s (Brooks, 1974), while the cyclodienes followed slightly later. The earliest herbicides (see below) were sodium chloride and chlorate, neither of which is selective. The introduction of selective herbicides, initially 2,4-dichlorophenoxyacetic acid (2,4-D), followed understanding of the indole-acetic acid system of auxins, which controls plant growth. The herbicides of the phenoxy acids group, which includes 2,4-D, are largely specific for dicotyledonous plants, sparing monocotyledons.

Classification and nomenclature

Classification

A major primary subdivision in the use of pesticides is into those used in agriculture and horticulture and those used in other situations, including non-agricultural, although some pesticides may be found in both major site use subdivisions. This overall primary subdivision may be reflected in the competent authorities responsible for the approvals processes. Thus, in the United Kingdom the approvals process for agricultural pesticides is managed by the Pesticides Safety Directorate at York, while non-agricultural pesticides are managed by the Health and Safety Executive (HSE) at Bootle. In the European Union a similar, but not identical, distinction is made between plant protection products and biocides. More detailed classifications of pesticides depend on the organism attacked (Table 1.1), according to chemical structure (Table 1.2), or according to mode of action (Table 1.3). However, and not for the purist, several practical schemes are an admixture of all the previous three, and usually on a combination of target organism (major division) and chemical class (subdivision).

Table 1.1 Classification of major pesticides according to the target organism

Pesticide type	Target
Insecticide	Insects
Fungicide	Fungi
Herbicide	Plants
Molluscicide	Slugs, snails
Rodenticide	Rodents
Acaricide	Mites
Nematicide	Nematode worms

Table 1.2 Examples of classification of pesticides according to chemical structure

Organophosphates
phosphates
phosphonates
phosphinates
phosphorothioates
phosphorodithioates
phosphoramides
phosphorothioamidates
Carbamates
N-methylcarbamates
dithiocarbamates
benzimidazoles
Organochlorines
Pyrethrum; synthetic pyrethroids
Phenols
Morpholines
Chloroalkylthiols
Organometallics
Azoles
Bipyridilium compounds
Ureas/thioureas
Anilines (substituted)
Chloronitrile

Table 1.3 Pesticide classification according to mode of action

Anticholinesterase (cholinesterase inhibitor)
Chitin synthesis inhibitor
Ecdysone agonist
GABA blocker (γ-amino butyric acid inhibitor)
Juvenile hormone analogues (insect growth regulators)
Anticoagulant
Glutamine synthetase inhibitor
Steroid demethylation (ergosterol biosynthesis) inhibitor
Protoporphyrinogen oxidase inhibitor
RNA-polymerase inhibitor
Thiol reactant
Protein synthesis inhibitor
Photosynthetic electron transport inhibitor
Mitochondrial respiration inhibitor

Nomenclature

The nomenclature of pesticides may be complex, but requires to be clearly understood and defined to avoid accidental misuse. The systematic chemical names are given according to the rules of the International Union of Pure and Applied Chemistry (the IUPAC name) and the Ninth Collective Index Period of the Chemical Abstract Service (the CAS name). Pesticides have, in addition to chemical names, national common names [e.g. British Standards Institution (BSI) and ISO (International Organization for Standardization)] (ISO, 1965, 1981). There are both English language and French language ISO names, and rules for translating ISO names into other languages such as Dutch. The English language ISO name is not always the same as the British or US common name, e.g. jodfenphos, whose British common name is iodofenphos. Pesticides will also have one or more trade names. Some pesticides are used as drugs in human or veterinary medicine and as such have international non-proprietary names (INNs). In some cases these may differ from the ISO pesticide names, e.g. imazalil, a pesticidal fungicide, is the same as the drug enilconazole. INN names are conferred by the World Health Organization (WHO, 2003). Additionally, pesticides will also be uniquely identified by a Chemical Abstracts Service (CAS) Registry Number and a Number (EEC number) in the European Inventory of Existing Chemical Substances (EINECS) or in the European List of Notified Chemicals (ELINCS). Under the CAS system, differing isomers, including stereoisomers, are given different Registry Numbers. For example, the (R) and (S) optical isomers, as well as the (RS) racemic mixture, and also the material of unidentified stereochemistry, will all have different numbers.

Exposure to pesticides; routes, monitoring, and protection

Routes and modes

There are many pathways by which humans can be exposed to pesticides. These are most conveniently and for practical purposes described for workers (occupational exposure) and the general public, although there are some clear overlaps (Table 1.4). These considerations indicate that the toxicity of pesticides by virtually all routes of exposure is relevant to human health, and this should be reflected in the toxicology testing requirements of the various pesticide safety precaution schemes.

Occupational exposure

Exposure occurs during the manufacture, transport, or use of pesticides, and relevant packaging and protective measures are necessary for all these situations. With over three million people being employed in farms in the United States, the

Table 1.4 Major exposure sources and routes

Source	Routes
Food residues	Oral
Water residues	Oral
Domestic/horticulture	Oral, percutaneous, inhalation
Public hygiene pesticide use	Oral, percutaneous, inhalation
Vector control	Oral, percutaneous, inhalation
Occupational exposure	Oral, percutaneous, inhalation
Human/veterinary medicine	Oral, percutaneous (inhalation)

potential for exposure is clear. Major occupational exposure routes are by direct contact of material with the skin, eyes, and respiratory tract. All these routes may be involved with airborne pesticide resulting from spraying or dust generation and the skin and eyes are principal routes for exposure to non-volatile liquids and solids that are not sprayed for application. Additionally, the alimentary tract may be a route of exposure from the swallowing of contaminated saliva or coughed mucus.

Skin

Contamination may occur from airborne material, from contaminated clothing and reuse of such clothing, during mixing, loading, application, harvesting, from foliar residues after re-entry into areas of sprayed crops, and from the handling of treated crops. Many studies have shown that workers exposed to pesticides may have residues on the skin. Occupational skin diseases are the second most common, representing about 30–45 per cent of all occupational illnesses. In California, 15–25 per cent of adverse pesticide reports to the state authorities are due to skin conditions (O'Malley, 1997). Increasing both environmental temperature and humidity may enhance the percutaneous absorption of pesticide on the skin, as may damage to the skin such as abrasions (Grissom and Shah, 1992; Maibach and Feldmann, 1974). Although cleansing the contaminated site is advised in order to remove residual pesticide, a simple soap and water wash may not be sufficient to do this, since it has been shown that washing the skin of pesticide or industrial chemical exposed humans or experimental animals may leave a considerable portion of the dose on, or in, the washed skin area (Webster and Maibach, 1983; Zendzian, 1989, 2003). Most of the residual material is to be found in the stratum corneum. Although there is a continual exfoliation of the corneum, the turnover time of the stratum corneum is of the order of 14 days (Halpron, 1972). Thus, there is a potential for the residual skin material to contribute to potential local and/or systemic toxicity. This may be compounded by the fact that washing the contaminated site can lead to a transient increase in the absorption flux of the material (Webster and Maibach, 1999). The timing and magnitude of any increase in absorption will vary with the specific chemical and its physicochemical properties, and the

rate/magnitude of absorption compared with the rate/magnitude of excretion of the absorbed chemical. In a detailed study Zendzian (2003) found that with 19 pesticides studied post-wash in the rat, absorption continued with 15 at all doses tested, in 2 continued but only at some doses, and with 2 volatile pesticides absorption did not continue post-wash. Although absorption from pesticide residue continued from washed skin with 15 pesticides, only with 9 was there an increase in systemic concentration, indicating a potential for increased toxicity. The finding of residual post-wash cutaneous pesticide indicates the need for studies on this effect to be added to percutaneous absorption investigations required for registration purposes (Zendzian, 1994).

Oral

Intake may result from swallowing of saliva contaminated from airborne material, and eating food or drinking water contaminated at work. Oral exposure may also result from transfer from contaminated hands, e.g. from eating or smoking.

Respiratory exposure

This occurs principally from material present in the atmosphere resulting from spraying or drift of pesticide. The atmospheric concentration will be affected by rate of application, type of formulation application (aerosol, dust), and meteorological conditions, principally air movement.

General public exposure

Although the general public appear to regard exposure to pesticides from residues in food and, perhaps, water of greatest concern, there are multiple other sources of exposure which can compound with those from residues. These include hand-to-mouth contact from pesticides used within buildings, veterinary medicines used against domestic pets (e.g. flea sprays), and contamination of food and working surfaces from the residential use of pesticides (e.g. control of insects). Thus, although the oral route is probably the major route of exposure for the general public, the skin and eyes probably are also significant, and inhalation the least. It should however be recognized that data on exposure by pathways other than food and drink is often very poor. An interesting intermediate between occupational and general population exposures is the 'take-home pathway', in which workers exposed to pesticides at work may take them back into their homes and contaminate members of the family. In one recent study (Thompson *et al.*, 2003), of 571 Washington state farm workers, mainly in fruit crops, 96 per cent reported exposure to pesticides at work. In a subset of respondents, pesticide levels above the limit of quantitation were discovered in the urine of children and adults and in house and vehicle dust. The results confirmed the existence of the take-home pathway of pesticide exposure, and accord with the fact that pesticides in soil and

house dust were significantly higher in the homes of agricultural workers compared with non-agricultural reference homes (Simcox, Fenske, and Wolz, 1995). It is notable that many employers did not provide resources for hand washing. This is clearly a route of exposure that requires more attention.

Monitoring for pesticide exposure

Occupational exposure

Skin

Pesticide exposure may be estimated by measuring residues on swabs taken from the skin surface, by hand rinses, by measuring residues on absorbent pads attached to clothing, or by measurements on removed samples of clothing. The surrogate skin techniques involve placing a collection medium against the skin or clothing and subsequently analysing for pesticide. The most common approach (patch technique) involves attaching patches (usually about 10) to clothing or directly to the skin; the chemical loading on the patch is extrapolated to the skin surface area. It is a simple method with some limitations, but does allow a semi-quantitative estimate to be made. Chemical removal techniques may be variable (Fenske, 1997). Dermal dosimetry techniques are available for research needs (Honeycutt *et al.*, 2001). Fluorescent tracer techniques can be used to qualitatively assess skin exposure, and can be combined with video imaging analysis to allow some degree of quantitation (Fenske, 1997).

As noted above there is a need to assess, ideally quantitatively, whether there is post-wash (decontamination) residue on or in the skin, since this may continue to contribute to toxicity. This may be done post-wash by chemical measurement of local skin residue, and/or following the absorption of the material by blood or plasma measurements of the labelled or unlabelled material (Zendzian, 2003).

Respiratory tract

Exposures can be estimated from measurements of concentrations in environmental air. This can be done by using passive or personal samplers (Griffith and Duncan, 1992).

General monitors for exposure

Biomonitors for the detection of over-exposure may be conducted as part of periodic medical examinations (see below). They may include Biological Exposure Indices (BEIs; ACGIH, 2002). BEIs and other biomonitors can involve several types of measurements of a chemical determinant; these include (a) analysis for the material or its metabolite(s) in body fluids (blood, urine, saliva) and sometimes hair, and (b) determination of the effects on a target molecule (e.g. haemoglobin alkylation or oxidation to methemoglobin) or enzyme (e.g. AChE) inhibition. These are more properly known as biomarkers of effect.

General public exposures

Exposure of the general population to pesticides through food can be estimated from measurements of residues in crops and foods. This can also be done by total diet studies, in which foods offered for sale are purchased in shops and analysed for various pesticides. In this way, geographical variations can be estimated, and account taken of total intake from various potential sources, including treated crops and from veterinary medicines in animal tissues.

Health issues

As was noted above, pesticides are a very large group of materials of many differing chemical structures (which may be used as one basis for classification) and consequently a wide range of potential interactions with biological molecules and cellular structures. It is not unexpected, therefore, that across the wide range of pesticide classes many differing types of local and systemic toxicity are seen. Hence the need for a detailed evaluation of the toxicity of individual pesticides, and also careful industrial hygiene and follow-up occupational medical surveillance programmes. The types of adverse health effects that have been documented in exposed workers with pesticides as a group have included acute toxic effects (mirroring the mechanism of toxic action), primary irritancy (the skin and eyes), sensitization (mainly allergic contact dermatitis, but on occasion respiratory sensitization as been described), peripheral and central neurotoxic effects, and myonecrosis (Baldi *et al.*, 1998, 2003; Langer *et al.*, 2003; Stallones and Beseler, 2002). In toxicology testing, and on occasion in epidemiology studies, there have been suggestions of cardiovascular toxicity, reproductive and developmental toxicity, endocrine oncogenicity, and immunotoxic effects (Al Thani *et al.*, 2003; Mathur *et al.*, 2002; Mills and Yang, 2003; Ritchie *et al.*, 2003; Settimi *et al.*, 2003; Sever, Arbuckle, and Sweeney, 1997; Zahm, Ward, and Blair, 1997). It is important to note that some adverse effects may be caused by impurities, and hence the importance to be aware, in detail, of the composition of the technical (in-use) material.

Factors specific to the toxicity of pesticides

As very disparate groups of chemical compounds, the toxicity of pesticides is very varied, both quantitatively and qualitatively. One aspect of pesticides that is of interest to the toxicologist is that in many cases their mammalian toxicology can be inferred from the mode of pesticidal action in the target species. Thus, many insecticides kill insects by effects on the nervous system, with specificity for the insects compared to non-target organisms being achieved by the relative accessibility of the insect's nervous system. Nevertheless, in sufficient doses, such insecticides may have similar effects on similar macromolecules in mammals to those that are targeted in insects. Examples of this include the anti-ChE OPs and carbamates, the synthetic pyrethroids, and organochlorines. In the case of fungicides, many affect steroid synthesis in both

fungi and mammals, e.g. the azole group of fungicides. On the other hand, it is sometimes possible to design pesticides which interfere with systems or metabolic pathways in target species, which do not exist in mammals. Examples among insecticides include chitin synthesis inhibitors and juvenile hormone analogs. In the case of herbicides, glyphosate, remarkable for its low mammalian toxicity, competitively inhibits 5-enoylshikimate 3-phosphate synthase, an enzyme in the shikimic acid pathway, required for the biosynthesis of phenylalanine, tyrosine, and tryprophan in plants. This metabolic pathway is not found in insects, birds, and mammals, conferring a very high degree of specificity on glyphosate. However, the fact that a biological system in the target species is not present in the non-target species does not always predict low toxicity, as is shown by the phenoxy herbicides. The auxin system of plant growth regulation is the target of the phenoxy herbicides, and this system is not present in mammals. Nevertheless, the phenoxy herbicides do have toxic effects in mammals. Furthermore, the technical product (which may itself contain impurities) is dissolved in solvents and co-formulants may also be present. Whilst the toxicological requirements for approval tend to concentrate on the active ingredient, in the case of pesticides of very low toxicity, such as glyphosate, the co-formulants may contribute substantially to the overall toxicity of the formulation.

Toxicological data requirements

In view of the wide range of potential acute and repeated exposure adverse effects, the large number of exposed and potentially exposed individuals (notably workers, incidental handlers, and consumers of treated crops), it is necessary to have a very detailed evaluation of the toxicology of a pesticide before it is approved for sale and use. Follow-up studies may be required if suggestions for unpredicted and unpredictable effects appear during post-approval use. The following list is intended only to illustrate the types of studies generically required by competent authorities in order to assess the potential adverse effects of a pesticide under the differing conditions of use. Each specific pesticide requires to be considered, case by case, based on factors that include the chemistry and physical properties of the material, known or suspect toxicology of the chemical group, and intended use pattern. Particular attention needs to be paid to the possible influence of the formulation, and the presence of impurities in the technical material.

Acute (single dose) studies. These are necessary to determine the lethal toxicity (LD_{50} and timed LC_{50}) and sublethal toxicity by all possible routes of exposure. They are needed in at least two species, and usually are required by peroral dosing, occluded cutaneous application, and inhalation. For baseline data, it may be necessary to have information by intravenous or intraperitoneal dosing.

Primary irritancy (inflammation). The skin and eyes are common sites of contacts and causes for occupational illness, and primary irritancy testing is required.

Sensitization. Because of the widespread use and potential for skin contact, studies on the likelihood for skin sensitization are required with all pesticides. Some preliminary information may be obtained by short-term *in vivo* and *in vitro* studies (Hermansky, 1999). The need for respiratory sensitization studies will be determined by the chemistry of the material and known occupational health effects.

Repeated exposure studies. To assess the potential for cumulative toxicity, repeated dosing studies are clearly needed from both the occupational and consumer perspectives. Again, because of the possible routes of exposure such studies usually are required by subchronic cutaneous application and peroral dosing. Depending on the formulation and mode of application, subchronic inhalation studies may be required. The need for combined chronic toxicity and oncogenicity studies will be determined by numerous factors including the chemistry of the material, residues data, known toxicity including genetic toxicology, metabolism and toxicokinetic data, and formulation/impurity considerations. In general, however, and partly for sociopolitical reasons, most pesticides will require chronic toxicity/oncogenicity studies to be undertaken before unconditional clearance is granted.

Developmental and reproductive toxicity. Such data clearly are necessary as a consequence of the large proportion and wide spectrum of the population being exposed and potentially exposed.

Genetic toxicology studies. These are considered as essential to determine the potential for biological reactivity and also for genotoxic carcinogenesis.

Metabolism and toxicokinetic studies. The nature and proportion of metabolites may provide information relevant to the potential for toxicity. Also, quantitative data on the absorption, biodistribution, and elimination of parent pesticide and metabolites is important in both the design and interpretation of repeated exposure studies, assessment of the potential for cumulative toxicity, and in quantitative risk assessment.

Antidotal studies. These are conducted in order to determine the value and the efficacy of general and specific treatments for pesticides poisoning. They are, for example, of value in assessing the effectiveness of atropine and oximes in OP poisoning. Into this generic category can be incorporated studies to test the efficacy of procedures designed to prevent the accidental ingestion of the more toxic pesticides by humans or domestic animals. These have included the inclusion of taste repellents and/or emetics into the pesticide formulation (Houpt, Xgoda, and Stahlbaum, 1984). Often, particularly with pesticides without substantial acute toxicity, antidotal studies are not required.

Special studies. In addition to the general toxicology studies of the types outlined above, special studies may be required which are related to the specific pesticide, or

its use, or potential misuse, or its formulation. Additive or potentiating effects, or additional toxicity due to formulation, may occur to variable degrees. Also, the influence of impurities, particularly in the technical material, may require investigation. A few of the many and differing additional studies that could be required are given as examples below.

- *Neurotoxicity.* Because of the biological reactivity of pesticides, there is a potential for neurotoxicity, and this is known to occur with certain classes of pesticides, e.g. antiChE OPs, organomercurials, and chlorinated hydrocarbon insecticides (Baldi *et al.*, 1998; Ecobichon and Joy, 1994; Keifer and Mahurin, 1997). Neurotoxic effects may be detected in repeated exposure studies with careful clinical observations and appropriate peripheral and central neurohistopathological techniques. With some classes of pesticides specific tests have been developed, e.g. with OPs the neurotoxic potential can be detected with chickens, and rat models and assays can be used for neurotoxic esterase (NTE) activity (Beresford and Glees, 1963; Johnson, 1987, 1992; Soliman and Farmer, 1984; Soliman *et al.*, 1982; Veronesi, 1992). A recent development has been the requirement by some regulatory authorities for developmental neurotoxicity studies.

- *Specific enzyme studies.* These may be required because of the nature of the chemical and its generic biological reactivity. For example, the OPs are known to be inhibitors of AChE and NTE. The former enzyme is important from a practical point of view because of the value of its measurement as an indicator of occupational exposure, and its value in the diagnosis of cholinergic poisoning by OPs. NTE measurement is a useful indicator of the potential for OPs to cause delayed-onset polyneuropathy, which is initiated by the phosphorylation of NTE (Johnson, 1987, 1992; Senanayake and Karalliedde, 1992; Senanayake, de Silva, and Karalliedde, 1992). AChE studies can be incorporated into acute and repeated exposure studies, and definitive tests are available for NTE assay.

Details of toxicology testing can be found in Anderson and Conning (1993), Ballantyne, Marrs, and Syverson (1999), Hayes (2001), and Santone and Powis (1991).

Human health effects

Human adverse health effects are documented from (or should be documented from) carefully prepared case notes of single or group poisonings, results from the findings of forensic pathologists and toxicologists in fatal cases, the records and published work of Poison Control Centres, and formal epidemiological studies. To the latter can be added the newer techniques of geographic processes for the capture, storage, retrieval, analysis, and display of spatial data (Clarke, McLafferty, and Tempalski, 1996; Gunier *et al.*, 2001; Ward *et al.*, 2000). These information

systems, which are automated, can be effectively utilized to study regional and temporal variation in the incidence of human symptomatic pesticide exposures (Sudakin *et al.*, 2002).

Occupational medical and biological monitoring

The monitoring of agricultural workers by standard clinical methods and special investigations allows for the detection of over-exposure and the development of the earliest signs of the potential toxicity of a pesticide (see also above). Two types of biological monitoring need to be considered. (1) Monitoring of biomarker compounds [either the parent compound or metabolite(s)]. Examples of this include alkylphosphates used to monitor the body load of OP pesticides. These give no indication of any effect of the pesticide, only of the bodily burden. (2) Biomarkers of effect. These may be studies on target molecules such as enzymes or target organs (e.g. the liver). An important example of a biomarker of effect is the inhibition of blood or plasma acetylcholinesterase (AChE) activity for the detection of potential over-exposure or for the diagnosis of poisoning with OP anti-ChEs. Inhibition of plasma AChE is usually a reliable monitor for occupational over-exposure, and in cases of accidental or deliberate poisoning in the acute phase a low AChE activity (<50 per cent of normal) is diagnostic but is not directly related to the severity of poisoning. This is somewhat surprising as poisoning by OPs is caused by inhibition of neural AChE, the same gene product as the red cell enzyme, but of course the nervous system is less accessible than the red cell to inhibitor in most cases. Red cell AChE inhibition is not a useful indicator for the development of delayed-onset polyneuropathy, but may be a useful predictor of the overall prognosis of OP poisoning (Aygun *et al.*, 2002; Besser and Gumann, 1994; Johnson, 1987). Methods are available for biological monitoring of many differing kinds of pesticides including OPs, carbamates, dithiocarbamates, phenoxyacetates, quarternary ammonium compounds, coumarins, phenols, organochorines, and pyrethroids (Maroni *et al.*, 2001).

With some aspects of medical monitoring, the significance may not be completely clear. For example, several studies have been conducted in which the genotoxic (mutagenic and clastogenic) activity of body fluids from agrochemical workers has been determined, e.g. with various classes of pesticides, studies have detected chromatid/chromosome damage and exchange of sister chromatid material (Bolognesi *et al.*, 1993; Carbonelli *et al.*, 1993; Garaj-Vrhovac and Zeljezic, 2003; Jablonicka *et al.*, 1989; Kourakis *et al.*, 1992; Nehez, Berencsi, and Paldy, 1981; Rupta *et al.*, 1988) and DNA damage (Garaj-Vrhovac and Zeljezic, 2003; Ribas *et al.*, 1995). However, whilst the results of several positive tests may indicate genome damage in somatic and germ cells and therefore a potential adverse health hazard, it is not totally clear what the specific end-point diseases may be, although oncogenesis and reproductive effects may be an obvious consideration. The results clearly indicate that detailed studies on cytogenetics monitoring of pesticide-exposed individuals combined with long-term epidemiology need to be conducted.

Protective and precautionary measures

Programmes for the safe use of pesticides require to be specifically developed for any given situation, depending on the chemistry and biological reactivity of the materials used: its formulation; the timing, frequency, and magnitude of exposure; place of exposure (e.g. manufacturing establishment, transport, agricultural, horticultural, home, etc.); known potential for adverse effects; environment; and meteorology. However, all programmes for the safe handling of pesticides should cover, at least, the following factors (Ballantyne and Marrs, 1992).

1. ***Worker education and training.*** This is required to cover the potential hazards from exposure to pesticides, and the protective and precautionary measures needed to ensure safe working conditions. In addition to formal education and training sessions, workers should have access to the manufacturer's literature and the Material Safety Data Sheet (MSDS) for the material(s) handled.

2. ***Protective measures.*** These should cover both collective and personal protection.

   Collective measures are those that are put into operation to protect groups of individuals at a given work site. They will vary according to the specific conditions of work (production factory, distribution site and transport, indoor treatment areas, outdoor treatment areas), but include the following:

   - Instruction and training of new employees and periodic updating of existing employees. This needs to cover instruction in potential hazards from pesticides and how to recognize and avoid these; sources of exposure and contamination; sources of information (e.g. label and MSDS); and protective measures (clothing and equipment maintenance).

   - Engineering controls and maintenance of equipment to avoid, for example, physical accidents and leakage of chemicals.

   - Paying attention to workplace exposure guidelines such as threshold limit values (TLVs) for chemicals in the atmosphere (e.g. ACGIH, 2002). Government-mandated permitted exposure limits may also apply in some countries. For example, in the United States the Occupational Safety and Health Administration (OSHA) sets permissible exposure levels (PELs), and in the United Kingdom the maximum exposure limits (MELs) and occupational exposure standards (OES) are set under the Control of Substances Hazardous to Health (COSHH) Regulations by the Advisory Committee on Toxic Substances (ACTS) and Working Group on the Assessment of Toxic Chemicals (WATCH) of the Health and Safety Executive (HSE). Clearly, these are relevant to enclosed spaces (factories, greenhouses, etc.) but cannot be applied to outdoor work conditions. This aspect

of protection takes into account equipment maintenance to prevent or correct leaks, area cleaning, and ventilation.

- Maintenance of alarm systems, if these are available for the chemicals employed.

Personal protective measures are those procedures that apply to ensure that the individual worker is specifically protected from over-exposure, and include:

- The availability of facilities to ensure that, if necessary, the worker may decontaminate himself at the end of a work day, or in the event of an accident. This will include access to washing and showering facilities.

- Personal protective equipment may be required, and be mandated, for some working conditions and with particular pesticides. This may include protective clothing and gloves, footwear, and eye protection. Whilst face shields give protection of the facial skin, they give only partial protection of the eyes, and for full protection of the latter it is necessary to use goggles. The choice of a respirator requires professional guidance with respect to the type and usage. There is need for provision of training, medical examination for suitability to wear a respirator, maintainance of equipment, and respirator fit. These have been considered elsewhere (Ballantyne and Schwabe, 1981). It is necessary to ensure that the recommended protective practices are followed; for example, agricultural workers frequently do not wear protective gloves when they have been advised to (Webster and Maibach, 1985).

- Medical biomonitoring of the worker may be required periodically (see the discussion of medical surveillance below).

- Continual review, random audit, and any necessary modification of the protective mechanisms programme employed. It is important to inter-relate all protective measures, some of which may be potentially detrimental with other hazards for which they are not intended. For example, agricultural workers are encouraged to wear sunscreen in order to reduce their risk of developing skin neoplasms from intense ultraviolet (UV) radiation exposure. However, some UV absorbing chemicals can act as skin penetration enhancers, which could therefore increase the percutaneous absorption of pesticides and other agrochemicals (Morgan, Reed, and Finnin, 1998; Nakai and Chu, 1997). In one experimental study it was found that 6 of 9 sunscreens tested led to a significant enhancement of the cutaneous penetration of 2,4-D (Brand, Spalding, and Mueller, 2002). Therefore, careful choice of sunscreen is necessary for use during pesticide application.

3. ***Occupational medical surveillance.*** For new workers it is essential to have a preplacement medical examination to determine his or her suitability to work

with pesticides. This will include a medical history and general physical examination. This, and any special investigations, should cover the potential for allergic skin disease, neurological and liver disease, and routine haematology and clinical chemistry evaluations. For those working with anti-ChE OPs it is necessary to conduct measurement of blood AChE. For established agrochemical workers there should be provision for periodic medical examination to detect any early indications of known adverse health effects. These periodic examinations may frequently be combined with any required biomonitoring for exposure (see above).

Environmental and ecotoxicology

Clearly the use of pesticides in agricultural, horticultural, pest control, and domestic purposes may lead to the widespread distribution of pesticides and a potential for ecotoxic and adverse environmental effects. Thus, regulatory authorities need to examine the potential for such effects as part of their determination of the suitability and safety of pesticides in respect of a safety precaution (clearance) scheme. Thus, competent authorities will need standard test information on the following, at least, as a component of the clearance scheme. *Avian toxicity* to include acute LC_{50}, short-term repeated dietary toxicity, and reproductive toxicity. *Aquatic toxicity* studies should include timed LC_{50} values for various species, short-term repeated toxicity, $\log P_{ow}$, bioaccumulation and bioconcentration, reproductive toxicity, and chronic toxicology. Also under this general heading it may be required to supply information on tainting from human taste studies. *Additional information* may also be required on toxicity to domestic animals. Many schemes also require information on persistence, degradation, and transport in soil, plant residues and metabolites, phytotoxicity, toxicity to worms, bees, etc.

Regulation of pesticides

It is clear from the above brief overview that the use of pesticides may result in adverse health effects in humans who are occupationally exposed (during the manufacture, formulation, transport, treatment, and handling of treated crops), who consume treated crops, and who are exposed to pesticides used domestically; also there are possibilities for ecotoxic and environmental effects. It is, therefore, equally clear that there is a need for regulation of these biologically active materials. Countries have processes (schemes) that vary in detail with respect to administration, notification requirements, definition, and criteria for interpretation and acceptability with respect to safety precautions. It is unfortunate that some schemes are so obviously and heavily infiltrated with burocratic and legislative language that the major objectives of the scheme, notably those relating to health, may become

administratively obscured (conceptually and in practice). There are attempts afoot to harmonize approaches across major parts of the world (Chaffey *et al*., 1999), and although this is a praiseworthy effort there may be difficulties due to political influences and pressures. In spite of this there is, as might be anticipated, a common medico-scientific thread to the various different geographic schemes. Details of the various processes, submission requirements, expectations, and differences between various schemes are presented and discussed in other chapters of this book. However, as a generalization the notifier of a new pesticide (or its definitional equivalent in differing schemes) can expect to be required to submit at least the following for consideration by the competent authority:

- ***Specification of the active material.*** This includes chemical identity (CAS and other identification numbers; chemical names; synonyms), physicochemical properties, purity and impurities (of the technical product), mode of pesticidal action, analytical methods.
- ***Proposed usage data.*** Intended usage(s) with rates and times of applications, formulation(s), efficacy data.
- ***Toxicology studies.*** As detailed above.
- ***Human toxicology and occupational medical data.*** To include any clinical information, surveillance data, and antidotal studies.
- ***Residues data.*** From treated crops and food from time of harvesting period, over storage period, and after processing. This to include quantitative aspects, degradation and conversion products, plant metabolites, and reaction products.
- ***Ecotoxicology and environmental fate and behavior.*** As detailed above, together with the results of any field studies.
- ***Other relevant information.*** This may include information on authorizations for other uses and in other countries or registration areas.

The administrative process by which the above information is reviewed, and recommendations made, varies between different competent authorities. In many, the differing aspects of the information submitted is examined by separate subgroups of independent professionals in the respective specialist areas, who will make their discussions and conclusions available to a main (oversight) group (committee) for the competent authority. Based on these, and their own deliberations in the wider perspective of the intended use of the pesticide, the oversight committee will make their decisions and recommendations known to the notifier through the administration of the competent authority. The details vary from

scheme to scheme, but in differing verbage and depending on the original notification the decision of the competent authority may result in clearance for a field trial, limited clearance, provisional commercial clearance, or commercial clearance. The latter has no conditions, but the first three have conditional restrictions on the clearance and, after a specified period, the notifier has to report back with additional studies and information requested by the authority, and reconsideration for full clearance. In reaching a decision on whether clearance is granted, and the level of clearance, the competent authority takes the following, amongst other issues, into consideration: protection of the user, consumers of the treated crop/foodstuffs, livestock, wildlife, and the general environment (Ballantyne, 1975). Along with the recommended level of clearance they will also issue advice on the above matters, together with advice on classification, labelling, and packaging the product, protective and precautionary measures, re-entry standards, maximum residue levels (MRLs), and acceptable daily intakes (ADIs). Many of these and related aspects may be dealt with by other government departments, or international authoritative bodies, for example the assignment of MRLs and ADIs is one function of the Joint FAO/WHO Meeting on Pesticide Residues (JMPR). Guideline Values (GVs) for drinking water are published by the WHO. Toxicity classification (hazard ratings) are assigned by the EPA, the WHO, and the EC. Classifications with respect to evidence of carcinogenicity (currently Groups 1–4) are assigned and published by the International Agency for Research on Cancer (IARC) and by the American Conference of Governmental Industrial Hygienists (ACIGH) who also publish workplace exposure guidelines (TLVs and BEIs).

Recent changes in regulation

Major changes have been made in the regulation in both the European Union and United States in the past 15 years. Use of pesticides on edible crops and horticulture is controlled in the European Union by Directive 91/414 (EEC Council, 1991). Many other uses will be controlled under the Biocides Directive (98/8/EC) (European Parliament and Council, 1998). The systems established under these Directives are gradually harmonizing the pre-existing national systems.

In the United States, until recently, the regulation of pesticides has been similar to the regulation of these substances in the United Kingdom, although there are differences in detail. The main law that regulated pesticides was the Federal Insecticides, Fungicides and Rodenticides Act (FIFRA; USA, 1947). The 1996 Food Quality Protection Act (FQPA) (USA, 1996) in the United States made changes in the methodology used for risk assessment in that country and may eventually impact on the methods used for risk assessment in the United Kingdom. The FQPA mandated (a) the consideration of all sources of exposure, and (b) the consideration of simultaneous exposure to more than one pesticide. The former was termed *aggregate exposure*, and the latter *cumulative exposure*. As the default assumption is that pesticides with similar mechanisms of toxicity will act additively, identification

of groups of pesticides with a common mechanism of toxic action [common mechanism groups (CMGs)] is necessary. This in itself is a challenging scientific problem (see Fenner-Crisp, 2000). Having identified the members of the CMG it is necessary to allow in some way for the differing potencies of the CMG. Methods of doing this have included the Hazard Index (HI) approach. A draft aggregate and cumulative risk assessment for OP AChE has been carried out (US Environmental Protection Agency, 2001). In the United Kingdom, a working group of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) (COT, 2002) considered the problem of exposure to mixtures of chemicals, including mixtures of pesticides. The report suggested alterations in the way in which risk assessments were undertaken, and that the default assumption could be made that pesticides with similar mechanisms of toxicity would act additively. The report also made extensive research recommendations. Furthermore, the COT identified gaps in knowledge on exposure to pesticides especially through non-food pathways and made the point that the targeted nature of the UK surveillance programme was less than ideal for gleaning population-based estimates of pesticide intake.

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Part I

Insecticides

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2 Toxicology of Organochlorine Insecticides

Andrew G. Smith

Although the organochlorine insecticides were widely used in agriculture and malarial control programs from the 1940s to 1960s with dramatic benefits, they fell into disfavour because of their persistence in the environment, wildlife, and humans. Much of the literature is now quite old but of interest for both practical and fundamental reasons. The relatively low cost of these insecticides and unavailability of complete substitutes (particularly for DDT), however, ensure their continued use in a few countries but it seems likely that this will still decline (Turusov, Rakitsky, and Tomatis, 2002; Weinhold, 2001). The use of DDT is still a controversial topic and sometimes, political correctness may have contributed to lack of malarial control due to restriction of its use (Attaran and Maharaj, 2000; Attaran *et al.*, 2000; Curtis and Lines, 1999; Roberts, Manguin, and Mouchet, 2000; Ross, 2000; Tren and Bate, 2001). In 2001 the United Nations Development Programme agreed that DDT could be used in certain circumstances to protect against malaria where resistance to other vector controls had developed especially in the light of its continued effectiveness (Curtis, 2002).

For toxicological purposes the chlorinated hydrocarbon insecticides may be divided into five groups: lindane, cyclodienes and similar compounds, toxaphene, DDT and its analogues, and the caged structures mirex and chlordecone. In spite of some similarity of chemical structure and pharmacological effect, the individual insecticides within each group may differ in degree of toxicity and in their capacity for storage. Furthermore, toxicity and storage do not always vary in a parallel way. Methoxychlor is much less toxic and much less stored than DDT, whereas endrin, which is more toxic than dieldrin, is stored far less.

Overview

Symptoms

In general, the signs of poisoning produced by different organochlorine insecticides are similar, that is, expressions of neuronal hyperactivity. However, there are

differences between the effects of DDT and its analogues and many of the other chlorinated hydrocarbon insecticides. Not only is tremor characteristic of poisoning by DDT, but also the onset of poisoning by it occurs with easily detectable mild effects that progress gradually, but continuously, sometimes to the point of convulsions. In contrast, lindane, aldrin, dieldrin, endrin, and toxaphene often produce a convulsion as the first sign of injury. This happens not only in experimental animals but also in people, who sometimes reported that they experienced no symptoms of any kind prior until the initial fit. The cyclodienes endrin, dieldrin, and isobenzan appear to be among the most toxic to humans and perhaps >10-fold more acutely toxic than DDT (Joy, 1982).

The degree of stimulation of the nervous system appears to be related directly to the concentration of these insecticides in nerve tissue. Usually the effect is rapidly reversible in animals after either single or multiple doses. Recovery occurs when the concentration of the chlorinated hydrocarbon insecticide in the nervous system falls below a critical level. This does not necessarily imply a loss of the chemical from the body but rather redistribution to other tissues, such as fat.

Routes of absorption

All organochlorine insecticides can be absorbed through the skin as well as by the respiratory and oral routes, but the importance of dermal absorption varies greatly for the different compounds. This is partly because some of them, such as methoxychlor, have such a low toxicity that a small amount absorbed by any route is of no importance; more importantly, the efficiency of dermal absorption varies for the different insecticides. DDT is poorly absorbed from solutions by the skin, and the absorption of solid material is so poor that it is difficult or impossible to measure either the uptake of DDT or its effect. In contrast, even solid dieldrin, if finely ground, is absorbed effectively through the skin and is about half as toxic when applied dermally as when administered by mouth. The intestinal absorption of these insecticides is influenced by fibre and fat constituents of the diet as well as by the total food intake (Heath and Vandekar, 1964).

Distribution, metabolism, and excretion

Organochlorine insecticides have become infamous because of their tendency to accumulate in humans, animals, birds, and the general environment. After single or repeated doses, most of these chemicals eventually reach their highest concentrations in adipose tissue with somewhat lower levels in other organs with high contents of lipids such as adrenals. Although storage in fat can be partly explained by the lipophilicity of these insecticides, other factors such as structural elements of the chemical and competition between binding sites in tissues are important (Bickel, 1984). Another, perhaps more important factor, is the rate of metabolism and excretion. For instance, DDT and its primary metabolite DDE are stored in

adipose tissue of humans, whereas methoxychlor, which is metabolized much more rapidly, occurs only at very low levels in fat. This difference led to the increasing use of methoxychlor as an insecticide and a decline in the use of DDT. The isomers of hexachlorocyclohexane are stored to very different degrees in a pattern that is probably due to some extent to differential metabolism. Dieldrin is stored avidly whereas its isomer, endrin, is stored so little that it has been detected in patients only after acute exposure and not even in people employed in its manufacture. The unhindered *anti*-C-12 hydrogen in endrin makes this position far more susceptible to attack than any other position in either isomer (Bedford and Hutson, 1976; Cole, Klevay, and Zavon, 1970). In itself, storage of chemicals in adipose tissue can be viewed as a detoxification mechanism.

Chlorinated hydrocarbon insecticides can be metabolized by the microsomal cytochrome P-450 system to hydroxyl derivatives, perhaps with dehydrochlorination as observed for lindane, or by conversion to stable epoxides as in the case of the formation of dieldrin from endrin. The *O*-dealkylation of methoxychlor also involves a cytochrome P-450-mediated hydroxylation step. Other routes of metabolism involve conjugation with glutathione or the formation of glucuronides. Besides the liver and adipose tissue, these lipophilic chemicals can be particularly localized and cause toxicity in brain, kidney and, interestingly, adrenal tissue.

Parent insecticides usually are excreted either in the bile, or possibly through the intestinal wall, for faecal excretion. Metabolites are excreted in the urine if they are of relatively high polarity such as glucuronides. This may have involved resorption of conjugates from the intestinal tract and transport to the liver and kidney (enterohepatic circulation), followed by further metabolic transformations. Such would be the case for glutathione conjugates excreted in bile, some of which may be reabsorbed and converted to the mercapturates for urinary excretion.

An important consideration when discussing the excretion of chlorinated hydrocarbon insecticides is their presence in milk (Jensen, 1983). The lipid content of milk (3–5 per cent) and high blood flow to breast tissue can lead to considerable concentration of these chemicals compared with that in tissues. Mobilization of fat due to starvation or other reasons can release stored chlorinated hydrocarbon insecticides into the circulation, sometimes with marked toxicological effects. Relationships between induction, storage, and excretion of metabolites are rarely simple; studies of the induction of the metabolism of one pesticide by another and effects on toxicity are still in their infancy (COT, 2002).

Effects on the central nervous system

Considerable evidence suggests that the insecticides act by altering the electrophysiological and associated enzymatic properties of nerve cell membranes, causing a change in the kinetics of Na^+ and K^+ ion flow through the membrane. Disturbances of calcium transport or Ca^{2+}-ATPase activity may also be involved (Ishikawa,

Charalambous, and Matsumura, 1989; Smith, 1991; Woolley *et al.*, 1985). Most studies have been conducted with DDT, chlorodecone, and cyclodienes. Full explanations for the differences in the *in vivo* neurotoxic effects of these groups of insecticides are still not completely available. DDT appears to act particularly at the nerve axon by prolonging opening of the ion gates of the sodium channel (Ishikawa, Charalambous, and Matsumura, 1989), whereas cyclodienes, mirex, and lindane seem to act at presynaptic terminals. Lindane, toxaphene, and cyclodienes have been shown to mainly act at the γ-aminobutytric acid (GABA)-regulated chloride channel (Casida and Lawrence, 1985; Cole and Casida, 1986; Lawrence and Casida, 1984; Narahashi *et al.*, 1995). However, more recent studies have shown this is a simplified view and that complex interactions with subtype GABA receptors occur, especially the $GABA_A$ receptor (Huang and Casida, 1997; Ratra, Kamita, and Casida, 2001; Ratra *et al.*, 2002). DDT, mirex, and chlordecone have no effect. The convulsant actions of dieldrin and lindane may be mediated by effects on the hippocampus and other limbic structures (Gilbert, 1992, 1995; Gilbert and Mack, 1995; Swanson and Woolley, 1978). Non-convulsant doses of chlorinated hydrocarbon insecticides increase the susceptibility of animals to convulsions precipitated by many other poisons or by electroshock.

Changes in the biogenic amines often parallel the toxicity of chlorinated hydrocarbon insecticides, including the phenomenon of initial illness followed by clinical recovery, but the significance of these changes is not well understood. Fever may be a specific result of poisoning of the temperature control centre in the brain. In humans a high fever of sometimes late but sudden onset has been followed promptly by death. This has been observed in poisoning by lindane, dieldrin, and endrin. Fever may also accompany convulsions in humans simply because it may be difficult to dissipate heat when it is generated by violent activity.

Effects on the liver

DDT and other organochlorine insecticides cause marked changes in the livers of various rodents, and in some species, especially the mouse, these changes progress to tumours. The relationship of these tumours to possible induction of hepatocellular carcinoma in humans is obscure although the view that they are peculiar to rodents may not be justified since there have been few studies with other species (Smith, 1991). In mice the hepatocarcinogenicity has been demonstrated in several strains and shows a dose–response relationship. Increased tumour incidence (particularly lung adenomas) has also been reported in some other organs of mice. DDT can be hepatocarcinogenic to rats (Cabral *et al.*, 1982; Fitzhugh and Nelson, 1947; Rossi *et al.*, 1977) whereas results in hamsters, dogs, and monkeys are inconclusive (IARC, 1974). Chlorinated hydrocarbon insecticides are, in general, negative in mutagenicity tests (Wildemauwe *et al.*, 1983) but DDT, BHC, and the cyclodiene insecticides are efficient promoters of the actions of recognized potent hepatocarcinogens (Pereira *et al.*, 1982; Schulte-Hermann, 1985; Williams and

Numoto, 1984). The ability of these chemicals to cause tumours in the liver and promote those initiated by other carcinogens may be associated with the induction of microsomal and other enzyme systems.

The response of the rodent liver to DDT is similar to its response to moderate dosages of BHC, chlordane, dieldrin and toxaphene (see Smith, 1991) and phenobarbital (Walker, Thorpe, and Stevenson, 1973; Wright *et al.*, 1972). The earliest changes in liver cells of rodents administered DDT involve so much increase in the smooth endoplasmic reticulum of individual cells that they enlarge. Endoplasmic reticulum forms whorls that may have fat droplets at their centres. Enlargement and morphological change of the mitochondria increased the number of primary lysosomes, and atrophy of the Golgi body also occurred (Obuchowska and Pawlowska-Tochman, 1973). At least in their early stages, the changes in liver cells in rodents are reversible if dosage is discontinued soon enough but may be slow when the inducer is persistent (Fitzhugh, 1947; Kunz *et al.*, 1966; Smith, 1991; Wright *et al.*, 1972).

As far as it is known, the chlorinated hydrocarbon insecticides and phenobarbital do not produce in other animals besides rodents, to the same extent, the changes in the endoplasmic reticulum that may be associated with tumour formation (Smith, 1991; Stevenson *et al.*, 1999). In addition, all chlorinated hydrocarbon insecticides may be positive carcinogens in the mouse but not all cause tumours in rats despite similar induction of the endoplasmic reticulum. An epigenetic mechanism (whatever this really means) for the hepatic carcinogenicity of chlorinated hydrocarbon insecticides has become a common hypothesis; possibly there is a disruption in intercellular communication, perhaps leading to inhibition of exchange of growth inhibitors (Tsushimoto *et al.*, 1983; Warngard *et al.*, 1989; Zhong-Xiang *et al.*, 1986). How this would relate to induction of microsomal enzymes, if at all, is still not clear.

Reproductive influence

Many of the chlorinated insecticides have endocrine disrupting properties, at least *in vitro* or in animal systems, perhaps by acting as weak oestrogens (Cummings, 1997; Smith, 1991, 2001; Soto *et al.*, 1995). Although the atrophic effects of lindane on rat testes occur at high doses (Raizada *et al.*, 1980) some sex-linked behavioural effects occur at doses, e.g. with chlordane (Cassidy *et al.*, 1994), that are compatible with those found in the general population. Other chlorinated insecticides or their metabolites may act as androgen receptor antagonists, such as p,p'-DDE (Kelce *et al.*, 1995). This whole area is the subject of considerable debate and though at first sight it may be separate from the issue of carcinogenicity, the same mechanisms may be highly pertinent for humans in the risk analysis of low-level environmental exposure.

Some studies have implicated a chronic oxidative stress in mechanisms of teratological actions of organochlorine pesticides (Hassoun, Bagchi, and Stohs, 1996; Hassoun and Stohs, 1996a, 1996b; Hassoun *et al.*, 1993).

Evidence for toxicity in humans

Acute or chronic neurotoxicity after high exposure to the chlorinated insecticides has been reported on many occasions but it is the low-level chronic exposure that continues to cause concern, even following greatly reduced usage (Turusov, Rakitsky, and Tomatis, 2002; Van Wendel de Joode *et al.*, 2001; Weinhold, 2001). This is particularly true in the consideration of neurological impairment of infants that may be exposed through their mothers' milk and at doses/body weight significantly greater than adults consuming pesticide-containing food (Hardell, Lindstrom, and Van Bavel, 2002). Recent studies have linked DDE (a metabolite of DDT) with pre-term and low birth weight babies (Longnecker *et al.*, 2001).

The evidence is weak, but not totally negative, that chlorinated hydrocarbon insecticides cause cancer in humans; DDT has been studied the most, especially in relation to breast cancer (see Snedeker, 2001, for a detailed review). Although studies are continually reported, the evidence for carcinogenicity in humans has been contradictory (Snedeker, 2001; Wolff *et al.*, 2000a, 2000b) not only with respect to developing breast cancer but also to survival (Demers *et al.*, 2000; Hoyer *et al.*, 2000). No increase in the occurrence of tumours has been found in heavily exposed populations. This includes groups of workers who manufactured and formulated DDT, dieldrin, aldrin, endrin, chlordane, and heptachlor and who have been examined carefully for tumours (Smith, 1991, 2001). However, in animals many of these insecticides are good promoters of liver and breast cancer (Schulte-Hermann, 1985; Scribner and Mottet, 1981) initiated by established carcinogens and there are reports of poorly defined human use exposures linked to increased incidences (Cocco, 1997). Some of the areas of the world where DDT and lindane have been used in large quantities are also areas where there is a significant risk of hepatocellular carcinoma due to aflatoxin contamination of food or to carrying of the hepatitis B virus.

Lindane/hexachlorocyclohexane (HCH)

1,2,3,4,5,6-Hexachlorocyclohexane (HCH) has eight separable steric isomers, one of which (the α) exists in two enantiomorphic forms. Of the eight isomers, six (including the two mirror-image forms of the α isomer, plus the β, γ, δ, and ε isomers) are relatively stable, and they are the only ones commonly identified in technical HCH but the γ-isomer, lindane, accounted for the insecticidal properties (Figure 2.1). 'Benzene hexachloride', is a misnomer for 1,2,3,4,5,6-HCH but is a term that was widely used and should not be confused with hexachlorobenzene. Lindane has had many formulations as concentrates, aerosols, sprays, wetable powders, and wood preservatives. HCH and lindane have been of value in the control of grasshoppers, cotton insects, rice insects, wireworms, and other soil pests. Lindane has been used for the protection of seeds, for treatment of poultry and livestock, and household insects. The use of HCH on a number of crops was

Figure 2.1 The α, β, and γ isomers of hexachlorocyclohexane. The last is lindane

never practicable because the mixture imparts to the food a marked off-flavour. For humans, lindane has been used as a scabicide and pediculocide, usually as lotions, creams, and shampoos.

Toxicity to animals

Acute poisoning by lindane involves increased respiratory rate, restlessness accompanied by frequency of micturition, intermittent muscular spasms of the whole body, salivation, grinding of teeth and consequent bleeding from the mouth, backward movement with loss of balance and somersaulting, retraction of the head, convulsions, gasping and biting, and collapse and death usually within a day (Cameron *et al.*, 1945). For most species, the compound has a moderate acute oral

Table 2.1 Comparison of acute toxicities to rats of some chlorinated insecticides

Insecticide	LD_{50} (mg/kg)	
	Oral	Dermal
Lindane	91	500
Chlordane	430	530
Heptachlor	100	195
Aldrin	39	98
Dieldrin	46	90
Isobenzan	6.6	–
Endrin	7.5	15
Endosulfan	43	130
Toxaphene	90	1075
DDT	113–450	250–3000
Methoxychlor	6000	–
Chlordecone	125	2000

Values are examples from various studies of mainly male rats, but sex is not always specified. Application by oral and dermal routes varies to some degree with dosing vehicle (Gaines, 1960, 1969; Smith, 1991, 2001).

toxicity (Table 2.1) not greatly different from that of DDT but it is more readily absorbed by the skin and therefore is more toxic by the dermal route than DDT. In rats, the acute toxicity of the isomers of BHC decreases in the order $\gamma > \alpha > \delta > \beta$ (Woodard and Hagan, 1947) but the toxicity of repeated doses decreases in the order $\beta > \alpha > \gamma > \delta$ (Fitzhugh, Nelson, and Frawley, 1950). The long-term toxicity of the different isomers is directly related to their storage and inversely related to their rate of metabolism (Davidow and Frawley, 1951; Macholz *et al.*, 1986).

Absorption and distribution

Although the α, β, γ, and δ isomers of HCH are stored in the fat of rats and dogs, over 30 times more β than γ is stored at equivalent dosage levels (Davidow and Frawley, 1951). This explains why the β isomer is more toxic when administered repeatedly even though the γ isomer is more toxic when given as a single dose. The difference in storage is explained by differences in metabolism. α-Hexachlorocyclohexane also seems to accumulate more than lindane, especially in the brain (Eichler, Heupt, and Paul, 1983; Stein *et al.*, 1980). The storage of isomers of HCH can be less at higher than at lower dietary levels and the more rapid equilibrium and relatively less storage at higher dosages are consistent with a dosage-related induction of microsomal enzymes (Macholz and Kujawa, 1985; Smith, 1991).

Metabolism and excretion

Not surprisingly multiple lindane administrations will induce expression of a number of drug metabolism enzymes involved in its oxidation and excretion, such as cytochrome P-450 isoforms and glutathione transferases (Kraus, Gross, and Kloft, 1981; Kumar and Dwivedi, 1988; Wolff and Suber, 1986) that may be genetically variable and be responsible in mice for strain differences in toxicity (Liu and Morgan, 1986; Robinson *et al.*, 1975).

Isomers of HCH are metabolized by slightly different routes and the biotransformation of lindane in mammals alone is complex. Many of the products or intermediates, when given separately, are converted to other metabolites not usually detected during the metabolism of HCH isomers. Full details of the metabolism of lindane and related chemicals can be found elsewhere (Macholz and Kujawa, 1985; Smith, 1991). Metabolism involves not only phase I pathways, such as oxidation by cytochrome P-450, but also phase II pathways, such as conjugation of alcohol and phenol products to form glucuronides. Hydroxylations, epoxidations, *cis* and *trans*-dehydrochlorinations, isomerizations, and desaturations lead to a large number of chlorinated cyclohexanols, cyclohexenols, and phenols (Chadwick and Freal, 1972a, 1972b; Chadwick *et al.*, 1978b; Chadwick *et al.*, 1981; Chadwick *et al.*, 1987; Fitzloff and Pan, 1984; Fitzloff, Portig, and Stein, 1982; Stein, Protig, and Koransky, 1977; Tanaka, Kurihara, and Nakajima, 1977, 1979a, 1979b). Under anaerobic conditions lindane is dechlorinated to chlorobenzene and benzene

(Baker, Nelson, and Van Dyke, 1985). There is little evidence that lindane is converted to other HCH isomers or to hexachlorobenzene in rats (Chadwick and Copeland, 1985; Copeland and Chadwick, 1979). Most of the di- and trichlorophenyl mercapturates observed in the urine of lindane-treated rats arise by conjugation of hexachlorocyclohexenes and pentachlorocyclohexenes with glutathione, followed by dechlorination (Kurihara, Tanaka, and Nakajima, 1979; Portig *et al.*, 1979). Many of the alcohol and phenol metabolites are excreted as glucuronides or sulphates. The types and amounts of alcohols and phenols can be varied in rodents by a number of factors including age of the animals, fibre content of diet, obesity, strain, and inducers of drug metabolism enzymes (Chadwick *et al.*, 1978b; Chadwick *et al.*, 1981, 1986; Chadwick *et al.*, 1987; Copeland *et al.*, 1986; Liu and Morgan, 1986).

The metabolism of other isomers of HCH besides lindane has not been studied in as much detail as that of the γ-isomer but probably occurs by routes very similar to those described above for lindane (Smith, 1991).

Neurotoxicity and behaviour

The different isomers of HCH have opposite pharmacological actions. Lindane is a stimulant of the nervous system, causing violent epileptiform convulsions that are rapid in onset and generally followed by death or recovery within 24 h (Coper, Herken and Klempau, 1951; Joy, 1982; McNamara and Krop, 1948; van Asperen, 1954; Woolley, 1985; Woolley *et al.*, 1985). Lindane may also cause hypothermia and anorexia in rats (Aldegunde villar *et al.*, 1981; Camon *et al.*, 1988a; Woolley, 1985). The α, β, and δ isomers are mainly depressants of the nervous system. The β isomer produces lameness and a peculiar flaccidity of the entire musculature. Following subcutaneous injection of mice with α-HCH or δ-HCH, the onset of effects is delayed compared with that of the γ-isomer. Poisoning by α-HCH is characterized by tremors of the extremities and inability of the animals to make coordinated movements. In studies of the relationships between the observable effects of lindane and the level and time course of concentrations in blood and brain, a good correlation was observed between dosages and frequency of onset of tonic seizure, intensity, and lethality (Tussell, Engel, and Casida, 1977).

The main site of action of lindane, unlike that of DDT, appears to be at the synapse with both excitatory and inhibitory effects (Joy and Albertson, 1985). The possible effect of inhibition of Na^+, K^+-ATPases on Ca^{2+} extrusion still requires more study (Woolley *et al.*, 1985). These effects occur at concentrations of lindane greater than required for antagonism of the GABA–receptor complex and among different HCH isomers are not specific for the γ-isomer (Bondy and Halsall, 1988; Joy and Burns, 1988). The actions do not appear to be a consequence of direct inhibition (Kamijima and Casida, 2000; Magour, Maser, and Steffen, 1984). Some biochemical changes after exposures to lindane or HCH do not seem to have direct connections with toxicity (Smith, 1991). For instance, there has been interest in the

turnover of inositol phospholipids after the occupation of some cell surface receptors by agonists and the involvement of Ca^{2+} (Holian, Marchiarullo, and Stickle, 1984; Stark, Chuang, and Joy, 1987). Lindane does not act as a competitive inhibitor in enzyme systems which act on *myo*-inositol but that in a fairly specific manner it inhibits phosphatidylinositol synthase (Parries and Hokin-Neaverson, 1985). Whether these effects of lindane on phosphatidylinositol metabolism are of any importance *in vivo* is not clear.

An important finding was the demonstration that lindane binds specifically to the GABA–receptor–ionophore complex, probably in the cerebellum (Matsumura and Ghiasuddin, 1983). This results in the effects of GABA and GABAergic transmission being disturbed (Lawrence and Casida, 1984; Matsumura and Ghiasuddin, 1983). The degree of binding for lindane, other BHC isomers, dieldrin, and other chlorinated insecticides correlated with their acute toxicities and abilities to induce convulsions at similar dose levels (Abalis, Eldefrawi, and Eldefrawi, 1985; Casida and Lawrence, 1985; Cole and Casida, 1986; Lawrence and Casida, 1984) especially the $GABA_A$ receptor (Nagata and Narahashi, 1995; Ratra, Kamita, and Casida, 2001; Ratra *et al.*, 2002). In addition, lindane inhibits stereospecifically the GABA-induced ^{36}CI-influx into rat brain membrane microsacs, whereas β-BHC has no effect (Abalis, Eldefrawi, and Eldefrawi, 1986; Bloomquist and Soderlund, 1985). Diazepam blocks the anorexic and hypothermic effects of lindane, and this could be taken as evidence for action at this receptor (Woolley *et al.*, 1985). However, the protection by lindane against the convulsant properties of pentylenetetrazole, which also acts at the GABA–receptor-linked chloride channel, after the insecticide itself has disappeared, shows that the mechanism is highly complex (Fishman and Gianutsos, 1987; Vohland, Portig, and Stein, 1981). Although some work implied that impairment of the GABA–receptor–ionophore complex might not be involved in the neurotoxicity of lindane *in vivo*, other evidence suggested differently (Bloomquist, Adams, and Soderlund, 1986; Cattabeni, Pastorello, and Eli, 1983; Sunol *et al.*, 1989).

Many of the symptoms of lindane poisoning are similar to those of dysfunction of the hippocampus–limbic system and measurements of glucose uptake by the brain at convulsant doses of lindane show increases in the limbic regions (Camon *et al.*, 1988b; Woolley *et al.*, 1984). Long-term potentiation by lindane of the evoked potential elicited in the dentate gyrus after stimulation of the prepyriform cortex occurs when levels of the chemical should have greatly diminished (Woolley, 1985; Woolley *et al.*, 1985). Kindling is a sequence of changes resulting from repeated stimulation of a part of the limbic system, such as the amygdala. Progressively severe behavioural signs are observed in the rat, commencing with eye-closing or chewing and climaxing in clonic motor seizure after lindane (Joy, 1982, 1985; Joy and Albertson, 1985, 1988; Joy and Burns, 1988; Stark, Albertson, and Joy, 1986). α-HCH has no apparent effect, but the β-isomer results in some delayed rates of kindling acquisition. Exposure of neonates to lindane can greatly enhance their acquisition of kindling in adulthood (Albertson, Joy, and Stark, 1985). Further

studies have suggested that the granule cell population of the dentate gyrus becomes more excitable and it seems likely that the convulsant action of lindane is explicable by interaction with the GABA receptor (Joy and Albertson, 1985, 1987a, 1987b, 1988). Experiments with cultured neurons from newborn rat dorsal root ganglia supported the hypothesis that lindane *in vivo* inhibits the GABA–receptor-channel complex but also raised the possibility of multiple receptors (Ogata, Vogel, and Narahashi, 1988). Behavioural effects might be linked to the neurotoxicity phenomena described (Tilson, Shaw, and McLamb, 1987).

Mutation and carcinogenesis

In host-mediated tests in mice and in direct tests on indicator organisms, HCH was not mutagenic (Smith, 1991; Wildemauwe *et al.*, 1983; Wolff *et al.*, 1987). Other *in vitro* tests have shown little evidence of genotoxic or mutagenic activity of lindane either (IPCS, 1991, 1992; Iverson *et al.*, 1984). Rats are not susceptible to the tumorigenic effects of lindane and HCH (IPCS, 1991, 1992; Smith, 1991). On the other hand, mice seem to develop hepatic tumours in some strains after chronic exposure to lindane and the other isomers as well as technical HCH. The α-isomer may be more active than the others. In mice with a dominant mutation at the agouti locus that increases susceptibility to strain-specific spontaneous and chemically induced neoplasms, lindane appeared to act as a tumour promoter via a cellular proliferation mechanism, perhaps as with other pesticides, by a non-genotoxic mechanism mediated by inhibition of the gap–junction intercellular communication (Guan and Ruch, 1996). α-HCH, or technical HCH, acted as a promoter of rat liver tumours or foci previously initiated by carcinogens such as diethylnitrosamine (Munir, Rao, and Bhide, 1984; Pereira *et al.*, 1982; Schroter *et al.*, 1987; Schulte-Hermann, 1985; Schulte-Hermann and Parzefall, 1981). In contrast, lindane protected rats against aflatoxin B_1-induced liver tumours (Angsubhakorn *et al.*, 1989). The relevance of these findings to human exposure is questionable.

Some studies have implicated a chronic oxidative stress in mechanisms of teratological actions of organochlorine pesticides (Hassoun, Bagchi, and Stohs, 1996; Hassoun and Stohs, 1996a, 1996b; Hassoun *et al.*, 1993).

Effects on reproduction

Chronic administration of lindane affects breeding parameters in rats (Nashteyn and Leybovich, 1971; Trifonova, Gladenko, and Shulyak, 1970). Lindane and the β-isomer are weakly oestrogenic to female rats and mice (Raizada *et al.*, 1980; Van Velsen *et al.*, 1986) and the β-isomer, lindane, and technical HCH have adverse effects on the testes of rats and mice (Chowdhury, Venkatakrishna-Bhatt, and Gautam, 1987; Huang and Huang, 1987; Nigam *et al.*, 1979; Shivanandappa and Krishnakumari, 1983; Van Velsen *et al.*, 1986). Seminiferous tubules become atrophied. The reduced sexual behaviour of female rats given lindane at proestrus does

not seem to be mediated through oestrogenic or GABAegic actions but by some other anti-oestrogenic mechanism (Cooper *et al.*, 1989; Uphouse, 1987; Uphouse and Williams, 1989).

Human toxicity

Use experience

Most documented experience of HCH is with lindane or the technical product, HCH. Although HCH was explored as a possible anthelmintic agent it caused a burning sensation of the tongue (Klosa, 1950). Use against scabies has been standard and generally trouble-free (Smith, 1991) but its use is now greatly restricted (Weinhold, 2001). It may soon end up being banned worldwide for environmental reasons as well as concern over long-term, low-level exposure of people. Permethrin cream rinse gave slightly better results against head lice than lindane with possibly fewer dermal reactions (Bowerman *et al.*, 1987). However, lindane appears to be the better scabicide (Shacter, 1981). Because of the possible susceptibility of infants, pregnant women, and patients with highly excoriated skin other insecticides probably should be used in these circumstances. Lindane can be absorbed through the skin to an appreciable degree over a period of a few days (Feldmann and Maibach, 1970). *In vitro* experiments demonstrated that lindane in the epidermis was effectively extracted by human plasma protein (Menczel *et al.*, 1984). The effectiveness of washing is variable and can actually aid absorption (Lange, Nitzsche, and Zesch, 1981; Nitsche, 1984).

Surveys of workers have revealed a moderate number of mild, acute intoxications. However, the cases often show many of the elements found in occupational poisoning by dieldrin and aldrin (Smith, 1991). No significant neurophysiological, neuromuscular, or other poor health effects could be detected in a group of 60 workers producing lindane for 1–30 years (Baumann *et al.*, 1981; Brassow, Baumann, and Lehnert, 1981) but abnormal EEG patterns in some workers exposed to HCH have been reported (Muller, Macholz, and Knoll, 1981). Toxic symptoms were observed in 90 per cent of workers engaged in the manufacture of technical HCH, some of whom showed evidence of cardiac effects (Kashyap, 1986).

The majority of effort to prevent poisoning by HCH and/or lindane focused on their use in vaporizing devices leading to low-level exposure. The unregulated sale of these devices led to their use in probably millions of homes.

Accidental and intentional poisoning

Many accidental poisonings were of children who ate vaporizer pellets (Smith, 1991). The clinical course of fatal poisoning occurred in as little as 2 h after ingestion. At the peak of its use, the number of recorded deaths from HCH was very small averaging less than one a year in the United States (Smith, 1991). Non-fatal cases

of illness occurred when HCH or lindane was accidentally added to food or food supply. Initial difficulty included malaise, faintness, and dizziness followed by collapse and convulsions, sometimes accompanied by foaming at the mouth and biting the tongue, nausea, vomiting, severe cyanosis of the face and extremities, facial pallor, and ocular defects (Bambov, Chomakov, and Dimitrova, 1966; Khare *et al.*, 1977). A moderate rise in temperature may be the consequence of the convulsions but a high fever has been reported and may be a direct toxic action of the pesticide especially in children (see Smith, 1991). Poisoned individuals eventually return to normal health, but in some cases this may take some weeks.

The amount of HCH ingested in fatal poisonings seems to have been approximately 200 mg/kg or above but reports of prompt vomiting in some cases and variation in the ages of patients confounds exact estimations (Jaeger *et al.*, 1984; Smith, 1991). Convulsions may be observed at considerably lower doses (Klein-Natrop, Roder, and Kadner, 1970). Clinical chemistry, EEG patterns, and pathology following lindane poisoning or exposure are not diagnostic (Smith, 1991). A variety of the chlorinated phenols described under Metabolism and excretion, above, have been reported in the urine of acutely poisoned individuals or exposed workers and may be found as sulphates, glucuronides, and mercapturates (Angerer, Maass, and Heinrich, 1983; Drummond, Gillanders, and Wilson, 1988; Starr and Clifford, 1972).

Cyclodiene and related insecticides

The cyclodiene and related insecticides shown in Figure 2.2 have many similar modes of neurotoxic action, probably related to that of lindane, causing convulsions rather than the severe tremors usually characteristic of DDT. However, distinct differences exist in their storage and potency. More detailed discussion can be found in Smith (1991). Even stereoisomers can differ remarkably in their potency of action or storage. Dieldrin is less toxic than endrin but is stored in the human population whereas endrin is not. Because of their overall similarity the chlorinated cyclodienes and analogues will be discussed together despite any specific differences which will be highlighted under appropriate aspects of their toxicology. Consideration of the acute LD_{50} values in rats (Table 2.1) illustrates some of the differences in potency between these insecticides. Of the group, dieldrin is the most thoroughly investigated.

Uses and production

Chlordane, heptachlor, aldrin, dieldrin, endrin, and α-endosulfan have had many formulations and uses in both agriculture and in the household, but not always in pure form. Some pesticides, such as aldrin, used on the soil gave distinct flavours to foods made from crops treated with them. Dieldrin was also used as a residual spray in homes in the control of vectors of tropical diseases, mainly malaria. Although

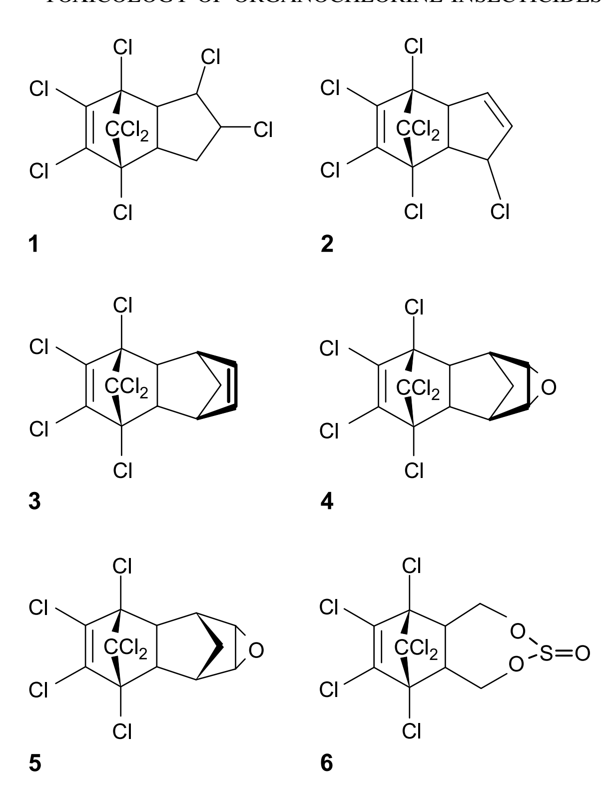


Figure 2.2 Some chlorinated insecticides of the cyclodiene type. 1, Chlordane. 2, Heptachlor. 3, Aldrin. 4, Dieldrin. 5, Endrin. 6, α-Endosulfan

developed as insecticides, it is interesting that endrin, perhaps the most potent of its class, was sometimes used to control voles and mice in orchards (Smith, 1991). The various insecticides of this class often had similar uses but were produced by different manufacturers. Probably aldrin and dieldrin were the most commonly used of the class not only for agricultural purposes but also in public health. The decline in their use followed the recognition that dieldrin, especially, was accumulating in wildlife up through the food chain with possible disastrous consequences.

Toxicity to animals

A comparison of the acute LD_{50}s of chlordane, hepatachlor, aldrin, dieldrin, endrin, and endosulfan are shown in Table 2.1. Although there is a wide range of potency from chlordane to endrin, all members of this group are toxic by the dermal route to an appreciable degree when compared with the oral toxicity. The exact values vary between investigators, sex, and vehicle used for dosing (Smith, 1991). Recovery can

be rapid in those animals that survive acute toxicity. With some insecticides, such as heptachlor and aldrin, the toxicities observed are probably due in part to their metabolism to the respective epoxides, e.g. heptachlor epoxide and dieldrin (Smith, 1991; Sperling, Ewenike, and Farber, 1972). Chronic toxicity of the cyclodiene-type insecticides is a balance between detoxifying metabolism and accumulation to critical levels enough to cause convulsions or, at lower accumulations, to give cause changes. A dosage of about 0.35 mg kg^{-1} day^{-1} of heptachlor in the rat was found in a two-year study to give hepatic changes whereas a dose of 0.25 mg kg^{-1} day^{-1} was a no observed adverse effect level (NOAEL) (Eisler, 1968). Dieldrin, and perhaps some of the other chemicals, cause liver tumours after prolonged dietary exposure (see below). For most of the chlorinated cyclodienes feeding or dosing of dams with the insecticide can cause increased mortality and decrease the number of offspring with perhaps skeletal abnormalities of the fetuses (Smith, 1991). Aldrin has been reported to cause kidney damage in rats and dogs (Reuber, 1980; Treon and Cleveland, 1955) and dieldrin to induce adrenal enlargement and functional changes (Foster, 1968). Both dieldrin and chlordane can alter the immune competence in mice. These changes with dieldrin can be dose dependent but may act through suppression of macrophage function (Barnett *et al.*, 1985; Loose, 1982). There seems little specificity in these findings that is pertinent to the toxicology for humans at likely exposure levels.

Absorption, metabolism, and excretion

All of these insecticides are efficiently absorbed from the intestinal tract, especially when dissolved in peanut or corn oil, possibly via the hepatic portal vein rather than via the lymphatic system as also occurs with DDT and hexachlorobenzene (Mueller *et al.*, 1978; Smith, 1991). What is particularly important is that unlike DDT they are also toxic by absorption through the skin. With dieldrin this even occurs with very finely ground powder (Hayes, Ferguson, and Cass, 1951). Because of the retention of dieldrin in the body, unlike some of its analogues, this insecticide can also be passed on to offspring during lactation.

Metabolism can both activate and detoxify these insecticides. Aldrin is metabolized by epoxidation to both *endo*-dieldrin and the *exo* isomer (the active dieldrin) by cytochrome P-450 isoforms (Newman and Guzelian, 1983; Wolff and Guengerich, 1987; Wolff *et al.*, 1980). In fact, epoxidation of aldrin has been used as an assay system for some cytochrome P-450 species. In extra-hepatic tissues such as seminal vesicles there is evidence for oxidation occurring by other mechanisms, perhaps catalysed by prostaglandin endoperoxide synthase (Lang, Frei, and Maier, 1986). As with the pairs aldrin and dieldrin, heptachlor and heptachlor epoxide, and isodrin and endrin (Eldefrawi *et al.*, 1985), it is probably the epoxide that is more toxic than its parent (Casida and Lawrence, 1985) although this may not be apparent from the LD_{50} values due to rapid metabolism to the epoxide *in vivo*. Metabolism of endrin, in turn, to *syn*-12-hydroxyendrin and then to 12-ketoendrin gives even greater potency (Table 2.2). In contrast to potentiation of action, metabolism of all these chlorinated

cyclodiene insecticides in the liver may lead to a variety of dechlorinated and hydroxylated products with a number of isomeric forms that can combine with cellular macromolecules and also be converted to glucuronides and sulphates for excretion. Some metabolites are stored more readily in the body organs than others. In mice *cis* and *trans*-chlordanes are less readily stored than the metabolite oxychlordane (Hirasawa and Takizawa, 1989). The metabolism of dieldrin has been studied in particular detail with rats, mice, monkeys, and other species. Some of the metabolites reported for the chlorinated cyclodiene-type insecticides are listed in Table 2.3.

Table 2.2 Microsomal metabolism of isodrin to endrin and further metabolites showing increased toxicity and greater affinity for the rat brain GABA receptor complex

	Acute toxicity LD_{50} (mg/kg)	Binding affinity IC_{50} (μM)
Isodrin	12–14	1.4
Endrin	8–43	0.22
syn-12-Hydroxyendrin	2–4	0.043
anti-12-Hydroxyendrin	>16	4.4
12-Ketoendrin	0.8–1.1	0.036

Data from Casida and Lawrence (1985).

Table 2.3 Some identified metabolites of cyclodiene chlorinated insecticides

Insecticide	Metabolite
Chlordane	*trans*-Chlordane
	Oxychlordane
	1,2-Dichlordene
	1-Hydroxy-2-chlordene
	1-Hydroxy-2-chloro-2,3-epoxy chlordene
Heptachlor	1-Chloro-3-hydroxychlordene
	1-Hydroxy-chlordene
	1-Hydroxy-2,3-epoxychlordene
Aldrin	Dieldrin
Dieldrin	6,7-*trans*-Dihydroaldrindiol
	Ketodieldrin pentachloroketone
	Hexachlorohexahydroindene-carboxylic acid
Endrin	*anti*-12-Hydroxyendrin
	syn-12-Hydroxyendrin
	12-Ketoendrin
Endosulfan	Sulphate derivative

Many of the hydroxylated products are conjugated as sulphates or glucuronides. Glutathione conjugation has not been studied in any detail (Smith, 1991).

Mutagenesis and cancer

Various studies show slight activity in mutational or chromosomal aberration assays and even formation of DNA adducts, but this seems to have little bearing on the development of tumours. With endosulfan, DNA adducts have been observed in cultured hepatic cells and parallels CYP3A induction (Dubois *et al.*, 1996), although it has been concluded that the pesticide has no carcinogenic potential (Hack, Ebert, and Leist, 1995). Some of the insecticides, including chlordane and dieldrin, cause liver tumours in mice after prolonged administration in the diet. This presumably occurs by non-genotoxic mechanisms and the evidence suggests that rats are less susceptible (Smith, 1991). Changes in liver histology with hypertrophy and induction of microsomal cytochrome P-450 activities are observed at quite low doses, e.g. 10 ppm in the diet of dieldrin, but it is equivocable whether there are any associated increases in the rate of liver tumour induction. For some of these insecticides, e.g. endrin, it is possible that neurotoxicity occurs with lower doses than that required to accumulate the body burdens necessary for liver tumour development. There is a marked formation of Mallory bodies in the liver tumours of mice fed dieldrin (Meierhenry *et al.*, 1983) possibly by hepatocyte premature ageing and polyploidization perhaps involving an oxidative stress mechanism (Klaunig *et al.*, 1995; van Ravenswaay, Toussaint, and Schmitt, 1988). Other studies have implicated inhibition of intercellular communication, as suggested for a variety of pesticides causing tumours (Zhong-Xiang, 1986).

Neurotoxicity

The neurotoxic action of dieldrin on the central nervous system typifies the neurotoxicities of the chlorinated cyclodiene-type insecticides. A major site of action appears to be at the synapse. Dieldrin binds to the picrotoxin binding site of the GABA–receptor–ionophore complex (Abalis, Eldefrawi, and Eldefrawi, 1985; Casida and Lawrence, 1985; Cole and Casida, 1986; Eldefrawi *et al.*, 1985; Lawrence and Casida, 1984; Matsumura and Ghiasuddin, 1983). This seems to be a similar mechanism for endrin and endosulfan. The degree of binding seems to correlate with acute toxicity (Nagata and Narahashi, 1994) and with convulsions, although other studies have been interpreted as not completely consistent with this explanation (Joy and Albertson, 1985). The exact brain location is probably similar to that demonstrated in the studies with lindane (Woolley *et al.*, 1985) as the synaptic processes of the thalamocortical relay. Parallel increases in blood flow and evoked response amplitude have been seen in rat cerebral cortex seizures initiated by dieldrin (Ray, Lister, and Roy, 1986). Transient hypothermia after a large dose of dieldrin has been reported and a reduction in food intake described (Woolley *et al.*, 1985) but it is not known whether this occurs by the GABAergic pathway. Although changes in various amino acids and amines of the brain have been shown following toxicity by these insectides it is difficult to explain these

mechanistically. Endosulfan appears to increase aggressive and locomotive behaviour in rats possibly by the serotonergic system (Agrawal *et al.*, 1983; Anand *et al.*, 1985; Seth *et al.*, 1986).

Human toxicity

Reported poisoning of people by the chlorinated cyclodiene insecticides has varied depending on the insecticide concerned. Few cases have been reported for chlordane, heptachlor, isobenzan, and endosulfan, whereas there have been a significant number of deaths from aldrin, dieldrin, and endrin, either accidental or suicidal (Smith, 1991). Frequently, despite convulsions, accidental poisonings by dieldrin, for instance, have not proved fatal. Many instances of poisoning by endrin, aldrin, and dieldrin have occurred by consumption of contaminated sugar, rice, seed grain, or bread made from contaminated flour in Africa and India. Sometimes this has been the result of contamination due to use of the insecticides near food, but in other instances no explanation for the route was ever found. Severe poisoning by endrin involves repeated, violent, epileptiform convulsions lasting a few minutes followed by semiconsciousness or coma until the next fit occurs, perhaps 15 or 30 min later. Even in moderate poisoning there might be no warning before the first fit especially in children. However, in a poisoning episode in Pakistan, thought to be due to endrin, older patients complained of headaches, nausea, and minor muscular spasms perhaps 30 min before collapsing (Rowley *et al.*, 1987). Serious poisoning can be accompanied by hyperthermia. Acute poisonings by dieldrin and chlordane seem to have similar symptoms although there may be small differences between the insecticides (see Smith, 1991). Cerebral and pulmonary oedema has been observed following a fatal dose of dieldrin (Steentoft, 1979). Dermal exposure can be as toxic as oral ingestion (Derbes *et al.*, 1955). Non-fatal, non-convulsant doses of chlordane, aldrin, endrin, or dieldrin have been associated with headaches, dizziness, gastrointestinal symptoms, skin irritation, and weakness in the legs. Some patients may experience temporary deafness.

Workers making or using these pesticides have shown the non-convulsant symptoms seen in acute poisonings. Workers heavily exposed to some chlorinated cyclodiene-type pesticides (e.g. dieldrin) have developed convulsions or hyperexcitability (Smith, 1991). With dieldrin a non-specific dermatitis has been reported, but this is not explained (Ross, 1964). Recovery may depend on which of the insecticides is responsible. Dieldrin may have a much longer half-life in humans than say endrin. Thus recurrence of convulsions may be more likely even though the insecticide is less toxic. There is no firm evidence that exposure to these pesticides increases cancer risks, but it has recently been proposed that dieldrin may influence survival from breast cancer (Hoyer *et al.*, 2000). It may be significant that heavy agricultural exposure to pesticides, particularly endrin, has been linked to an excess of deaths due to stroke and a significant excess of deaths from cerebrovascular diseases has been noted (Loevinsohn, 1987; Shindell and Ulrich, 1986).

Toxaphene

Extensive chlorination of camphene to 67–69 per cent by weight gives a mixture of many chemicals known as toxaphene, which has been used as an insecticide. During the chlorination procedure 2-*exo*,10-dichlorobornane is first formed by rearrangement so that many of the products are bornanes not camphenes. Three of the main products identified as significantly accounting for toxicity are shown in Figure 2.3. A similar product has been formed by the chlorination of pinene. Toxaphene was valued both for its power as an insecticide in a variety of formulations and also because of its limited environmental persistence and rapid excretion by mammals (Smith, 1991). Studies of its metabolism have been difficult because of the complicated mixture involved. Following initial distribution throughout the body of rats, redistribution to adipose tissue occurs (Wen and Chan, 2000) and persistent labelling of the adrenals (Mohammed *et al.*, 1985). However, extensive metabolism by dechlorination seems to occur (Pollock and Kilgore, 1980) probably with conjugation to glucuronic acid and glutathione; levels in tissues can decline rapidly (Smith, 1991).

Toxicity to animals

The acute toxicity of toxaphene is similar to that observed with lindane and the cyclodiene-type chlorinated insecticides. Animals show hyperactivity, muscle spasms, convulsions, and coma but may recover quickly with metabolism of the toxaphene. As can be seen from Table 2.1, toxaphene is moderately toxic to rats by mouth but substantially less toxic dermally, unlike endrin and dieldrin. One of the

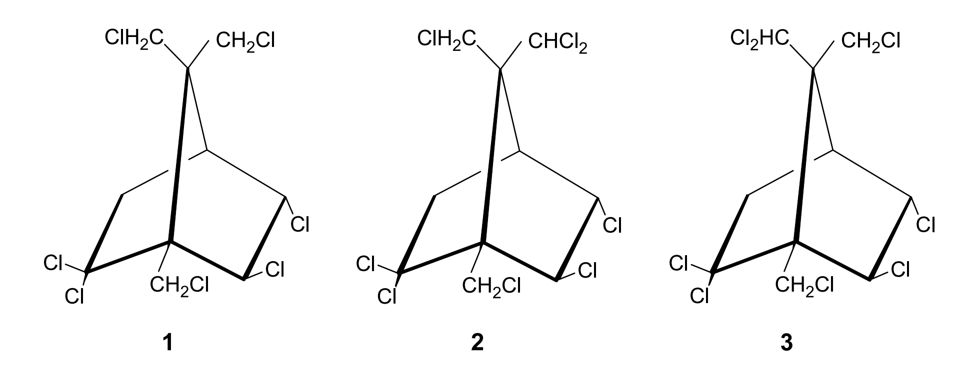


Figure 2.3 Some polychlorobornane constituents of toxaphene which account significantly for the acute toxicity of the mixture (Smith, 1991). 1, Toxicant B. 8-Cl Toxicant B. 3, 9-Cl Toxicant B.

problems of assessing the toxicity of such a mixture is the variability of response depending on the source. In mice, the 8-chloro-B constituent seems to be the most acute orally toxic constituent (Turner, Engel, and Casida, 1977).

Toxaphene constituents have been mutagenic in some tests but did not give a response in the mouse-dominant lethal assay (Goodman *et al.*, 2000). As with many of these chlorinated chemicals, prolonged feeding can cause liver and thyroid tumors (NCI, 1979) but probably not by a genotoxic mechanism since no adducts are observed (Hedli *et al.,* 1996). Thyroid tumours are probably the result of thyroid stimulating hormone driven proliferation (Waritz *et al.*, 1996). The influence of toxaphene on reproduction is mild (Smith, 1991). Like the other chlorinated insecticides, there have been biochemical studies on various ATPase functions following toxaphene poisoning in animals, but it is difficult to put these in context (Smith, 1991). Perhaps the most important studies are those showing that the 9-chloro toxicant B is a potent ligand for the picrotoxin binding site of mouse brain synaptosomes blocking the GABA-regulated chloride ionophore (Casida and Lawrence, 1985; Cole and Casida, 1986; Lawrence and Casida, 1984). Exposure of cynomolgus monkeys to varying levels of toxaphene (up to 0.8 mg kg^{-1} day^{-1}) for 20 weeks showed decreased serum cholesterol levels but no other detectable changes (Arnold *et al.*, 2001).

Toxicity to humans

Fatal and non-fatal acute poisonings by toxaphene begin within 30 min of ingestion of the pesticide and are characterized by mental confusion, possible nausea, jerking of arms and legs, and by convulsions. In many cases of survivors, recovery was rapid (Smith, 1991). Chronic exposure to chlorinated camphene and pine mixtures similar to toxphene apparently causes headache, nausea, and abdominal pain and weakness (Bezuglyi and Mukhtarova, 1969; Smith, 1991). Exposure to these products has also been reported to cause chromosomal abnormalities of lymphocytes (Samosh, 1981). What little is documented suggests that a fatal oral dose to humans is about 10 mg/kg (McGee, Reed, and Fleming, 1952).

DDT and its analogues

Although DDT was synthesized by Zeidler in 1874, its insecticidal properties were not discovered until 1939. After testing in the United States and the United Kingdom it was used to great effect in the Second World War for controlling typhus and malaria both in populations and troops. The importance of DDT to the war effort after 1943 was as important as penicillin. DDT first became available for civilian use immediately after the war had ended and it was immediately applied to not only controlling malaria but for other insect-transmitted diseases. The use

of DDT was a major reason for the eradication of malaria in the United States and many parts of Europe. The amount of DDT used in these capacities was relatively small (Hayes, 1991). Its spectacular success encouraged its use in general agriculture to protect production of economically important crops. Even its public health use in combating days of labour lost to malaria had huge economic benefits, for instance in India. It has been estimated that the number of lives saved by DDT runs into millions (Hayes, 1991). However, it was soon recognized that DDT and its metabolite DDE could accumulate in animals and people and there was concern about the health repercussions to wildlife and humans. In addition, resistance of some insect populations started to be observed. From this point the agricultural use of DDT declined although there has been a continued, vital niche for it in malaria control despite attempts to have it banned worldwide (Attaran and Maharaj, 2000; Attaran *et al.*, 2000; Curtis and Lines, 1999; Roberts, Manguin, and Mouchet, 2000; Ross, 2000). The knowledge that traces of it are stored in essentially everyone in the world has kept DDT in the political spotlight. Under these circumstances it is not surprising that DDT has been studied more thoroughly than most pesticides. Analogues of DDT have been produced, such as ethylan, chlorobenzilate, dicofol, and methoxychlor (Figure 2.4), in order to decrease particular side-effects or to make the compound effective but easily metabolized and of least threat to the environment.

Figure 2.4 DDT and some analogues. 1, *p,p′*-DDT. 2, Methoxychlor. 3, TDE (*p,p′*-DDD). 4, Dicofol. 5, Ethylan

DDT

p,p'-DDT, is 1,1′-(2,2,2-trichloroethylidene)-*bis*(4-chlorobenzene) and is an acronym for *d*ichloro*d*iphenyl*t*richloroethane. DDT has been sold under a variety of trade names (see Smith, 2001). Technical DDT contained p,p'-DDT, 77.1 per cent and o,p'-DDT, 14.9 per cent p,p'-DDD, 0.3 per cent, o,p'-DDD, 0.1 per cent; p,p'-DDE, 4.0 per cent; o,p'-DDE, 0.1 per cent and was formulated in many ways.

Toxicity to laboratory animals

The first observed effect of DDT in rats is abnormal susceptibility to fear, with violent reaction to normally subthreshold stimuli. There is motor unrest and increased frequency of spontaneous movements. As poisoning progresses hyperirritability similar to that seen in strychnine poisoning develops but without the violent convulsions. A fine tremor is present intermittently without observable cause, and is finally present as a coarse tremor. In the later stages at high dose, in some species, there are attacks of epileptiform, tonic–clonic convulsions with opisthotonos. Symptoms appear several hours after oral administration of the compound, and death follows after 24–72 h. The latent period after intravenous administration at about the LD_{50} levels is approximately 5 min; signs of poisoning reach a maximal level in about 30 min, and survivors are symptom-free in 18–24 h. Poisoning by repeated doses of DDT differs from that of a single dose only insofar as the animal may be gradually debilitated, especially by malnutrition.

Dissolved DDT is absorbed by all routes, but DDT powder is very poorly absorbed through the skin. It is very difficult to put enough DDT dust on the skin of animals to kill them, so that an accurate LD_{50} value for this formulation cannot be determined by the dermal route. o,p-DDT seems to be appreciably less toxic, for most end points, than p,p'-DDT (Dale *et al.*, 1966). Those metabolites that have been tested seem to be less toxic than the parent compound (Smith, 2001). One exception is probably DDA, which seems to be toxic to the kidney. Young animals do not seem to be more susceptible than adults to the acute effects of DDT neither are there particular distinctions between sexes (Smith, 2001). Hamsters seem to be more resistant than rats to the effects of DDT. In chronic studies no effect levels have been reported of 0.05 mg kg^{-1} day^{-1} for the rat, 8 mg kg^{-1} day^{-1} for the dog, and 2.2–5.5 mg kg^{-1} day^{-1} for the monkey (Smith, 1991). At chronic doses to rodents equivalent to that reported for heavily exposed workers very slight increases in liver hypertrophy may be detected.

Absorption and distribution

Most DDT dust particles are of a size that, if inhaled, they are deposited in the upper respiratory tract and probably swallowed. Thus exposure to DDT via inhala-

tion is of minor importance compared with exposure by other routes. DDT dissolved in oils is absorbed from the gastrointestinal tract about 1.5–10 times more effectively than is undissolved DDT (Palin *et al.*, 1982). The initial distribution of DDT, like some other chlorinated chemicals, seems to occur via the lymph system (Pocock and Vost, 1974; Sieber, 1976). Most of the DDT absorbed into the lymph is carried in the lipid core of chylomicrons and thence into the plasma proteins. p,p'-DDT is taken up at a rate which is different from that of o,p'-DDT and does not strictly parallel differences in lipid solubility.

DDT is stored in all tissues and particularly high concentrations are found in fat. After repeated doses, storage of DDT in fat rises rapidly at first and then more gradually until a peak or plateau is reached. This may be much greater than is seen with a single fatal dose. Levels in the brain, whether from acute or chronic exposure, seem to be the more critical factor. Concentrations gradually decline once exposure ceases. Tissues in the fetus are lower than those in corresponding tissues of the mother. Most species, including humans, but perhaps excluding the rhesus monkey, store the metabolite DDE more tenaciously than they do DDT, the greater part of which is metabolized by a different pathway from that of DDA and excreted more rapidly. The result is that DDE, expressed as a percentage of total DDT-related compounds, increases in individuals after DDT intake decreases and increases in successive steps of the food chain (Smith, 2001).

Metabolism and excretion

The major metabolite of p,p'-DDT was recognized in early work as 2,2,-bis(4-chlorophenyl) acetic acid (DDA) and excreted in the urine. Similar metabolism was shown for o,p-DDT and seems to be common for many species, including humans. A simplified scheme of DDA formation from DDT is shown in Figure 2.5. The sequence of metabolism *in vivo* is likely to proceed via more than one pathway. There is evidence for both reductive and oxidative metabolism of the trichloroethane function involving the formation of either an acyl chloride intermediate or an aldehyde (Fawcett *et al.*, 1987; Gold and Brunk, 1983, 1984; Kujawa, Macholz, and Knoll, 1985). As with lindane a variety of minor metabolites are possible and have been identified in excreta (Smith, 2001). Another route of metabolism of DDT is by dehydrochlorination to form DDE (Smith, 2001). It is possible that this could be further metabolized via the acyl chloride route, but most of the metabolites of DDE identified have been hydroxylated analogues such as 4-hydroxy-p-DDE, 4-hydroxy-m,p-DDE, 3-hydroxy-p,p'-DDE, and 2-hydroxy-p,p'-DDE (see Figure 2.5). Hydroxylation of DDE to form some of these products probably occurs by epoxidations and in some cases NIH shift rearrangements (Gold and Brunk, 1986). There is evidence for glutathione conjugation of p,p'-DDE followed by conversion to a mercapurate, cleavage by a lyase, and then methylation of the thiol (see Smith, 2001). Similar products have been observed in the metabolism of chlorinated benzenes and biphenyls.

Figure 2.5 Simplified scheme of p,p'-DDT metabolism in rodents. 1, p,p'-DDE. 2, 4-Hydroxy-p,p'-DDE. 3, p,p'-DDD. 4, Putative acyl chloride intermediate. 5, p,p'-DD-aldehyde. 6, p,p'-DD-alcohol. 7, p,p'-DD-acid (DDA)

Metabolism of o,p'-DDT is similar in many ways to that of the p,p'-analogue but at a greater rate because of the ease of ring hydroxylation. Strong evidence seems to suggest that activation of o,p'-DDT or o,p'-DDD occurs by the action of cytochrome P-450 particularly in the adrenal mitochondria, possibly by the intermediacy of a reactive acyl chloride (Martz and Straw, 1980; Pohland and Counsell, 1985). Binding of o,p'-DDD in mouse lung and p,p-DDD in rabbit Clara and human bronchial epithelial cells may occur by a similar mechanism (Lund *et al.*, 1989; Nichols *et al.*, 1995). 3-Methylsulfonyl-p,p'-DDE is selectively bound and toxic to the adrenal zona fasciculate of mice, even occurring in the pups of dosed mothers (Jonsson *et al.*, 1992; Lund, Bergman, and Brandt, 1988). It is thought that the damage results from activation in the mitochondria by CYP11B (Jonsson, Rodriguez-Martinez, and Brandt, 1995; Jonsson *et al.*, 1991). Similar mechanisms may apply to the toxicity to the adrenals of o,p'-DDD, toxicity being greater because of the faster metabolism of the p,p'-analogue (see Smith, 2001).

Most of the metabolites of DDT are excreted via the bile and are either DDA or related metabolites. Importantly, the difference between rats and birds in the excretion of DDT metabolites seems to be the reduced ability of the latter to metabolize DDE further (Fawcett *et al.*, 1987). As with many lipophilic chlorinated chemicals, excretion of DDT in the milk can be an important clearance route for mothers in rodents, cows, and humans (Jensen, 1983; Smith, 1991, 2001).

Neurotoxicity and behaviour

The major effect of DDT clearly is on the nervous system, and this seems to occur by slowing down the closing of 'gates' in axon ion channels (Woolley, 1985). Both central and peripheral parts of the nervous system are affected although the cerebellum and motor cortex have been viewed to be of greatest importance. Electrical activity may become abnormal only a few minutes after a large dose of DDT to rats which can culminate in seizures (Joy, 1973). Unlike the cyclodienes or lindane, the most characteristic feature of DDT in rats is production of tremor. There also seems to be other disturbances of thermal regulation such as coldness of the skin. Death from DDT in experimental animals is usually from respiratory arrest often from a prolonged period of muscular activity leaving them exhausted. Some evidence supports the hypothesis that in particular circumstances and with large doses, DDT directly affects the myocardium as well as producing fibrillation induced via the central nervous system (CNS) (Smith, 1991). Behavioural changes have also been shown in animals receiving low doses of DDT, attributed to an exaggeration of the stretch reflex, without other apparent symptoms (Khairy, 1959). At a single dose of 12.5 mg/kg of DDT to rats there is a significant increase in the acoustic startle response that can be attenuated by phenytoin (Saitoh, Shaw, and Tilson, 1986; Tilson, Hudson, and Hong, 1986).

The effect of DDT appears to be on axonal membranes and the inhibition of Na^+, K^+, and Mg^{2+} ATPases and Ca^{2+}-ATPase regulating calcium levels at the axon surface (Ghiasuddin and Matsumura, 1979). DDT is known to prolong opening of the ion gates of the sodium channel, perhaps by affecting phosphorylation of the α-subunit, and interacting with the sodium channel of rat dorsal, but not ganglion, neurones in a similar manner to pyrethroids (Ishikawa, Charalambous, and Matsumura, 1989; Song *et al.*, 1996). Although spinal α-adrenoceptors have been proposed as modulating DDT-induced tremor, attenuation of motor dysfunction requires blockade at receptors in regions other than solely the spinal cord (Herr, Gallus, and Tilson, 1987; Herr, Mailman, and Tilson, 1989). Changes in metabolism of 5-hydroxytryptamine and norepinephrine and other messengers have been proposed as accounting for some of the neurotoxic effects of DDT, but it is difficult to put these findings into the context of brain and nerve micromorphology (Smith, 2001).

Mutation and carcinogenesis

Most *in vitro* and *in vivo* studies of the mutagenicity of DDT have given inconclusive results (Coulston, 1985). As with some other chlorinated aromatics, DDT causes inhibition of intercellular communication in rat and hamster cell lines (Flodstrom *et al.*, 1990; Tsushimoto *et al.*, 1983; Warngard *et al.*, 1989) and freeze-fracture of hepatocytes from rats administered DDT showed a reduction in the number and size of gap junctions (Sugie, Mori, and Takahashi, 1987).

Expression and turnover of connexins and some phosphorylated forms have been implicated to be involved in these processes (Ruch *et al.*, 1994; Tateno *et al.*, 1994). In *in vivo* studies high levels of DDT have been shown to induce liver tumours in rats and mice although these reports have not always been repeatable (Cabral *et al.*, 1982; Fitzhugh and Nelson, 1947; NCI, 1978; Rossi *et al.*, 1977; Turusov, Rakitsky, and Tomatis, 2002). Certainly DDT promotes carcinogenesis initiated by aflatoxin and other chemicals (Peraino *et al.*, 1975; Schulte-Hermann, 1985; Scribner and Mottet, 1981; Williams and Numoto, 1984). The significance of these findings for humans is open to question.

Influence on reproduction

Recent concern that chlorinated chemicals may act as endocrine disruptors has resurrected interest in early work on the oestrogenicity of DDT (see Smith, 1991). Some of the earlier studies on DDT oestrogenicity probably stemmed from the fact that the o,p'-analogue was present at significant quantities in the technical product. o,p'-DDT acts on the uterus probably as a long-lasting oestrogen receptor agonist, although it seems that its actions may not be identical to oestradiol (Galand *et al.*, 1987; Robison, Schmidt, and Stancel, 1985a; Robison, Sirbasku, and Stancel, 1985b). Relative to a potent chemical such as diethylstilboestrol, o,p'-DDT is about $1/10\,000$ less active so that at environmental exposure levels and the fact that it is metabolized faster than p,p'-DDT make it seem unlikely to be a major hazard in this respect. Recent evidence supports the view that the DDT metabolite p,p'-DDE can disrupt male reproductive development by acting as an antiandrogen by binding to androgen receptors in a non-productive way (Kelce *et al.*, 1995, 1997). DDE, like vinclozolin and flutamide, changed the expression of androgen receptor regulated genes in castrated male rats. Exposure of Long–Evans rats, but to less degree Sprague–Dawley, to DDE *in utero* by lactation can show changes in ano-genital distance but not testes, epididymus, seminal vesicles or ventral prostate weights and at maternal doses at or above 10 mg/kg. In Holtzman rats, effects on prostate development by *in utero* or lactational exposure to p,p'-DDE has been shown (Loeffler and Peterson, 1999; O'Connor *et al.*, 1999; You *et al.*, 1998). Investigations on the influence of DDT on reproduction, including multigeneration studies, have shown no clear effects in dogs, rats, and mice except at high overtly toxic doses (Smith, 2001).

General metabolic responses

p,p'-DDT, p,p'-DDE, o,p'-DDT, and various metabolites of these will cause a variety of metabolic changes to occur, principally in the liver. Foremost of these is the induction of microsomal enzymes. Studies are mainly old and this is reflected in the types of analyses performed (Smith, 2001). Multiple bi-daily doses of DDT to female rats induced hepatic CYP2B and 3A proteins but not CYP1A1 or 1E1

and caused elevated hydroxylation at positions 16 and 6β of testosterone (Li, Dehal, and Kupfer, 1995). DDT, DDE, and DDD all induced CYP2B and 3A in male rat liver, roughly to the same degree despite marked differences in retention (Nims *et al.*, 1998). Changes in lipid metabolism that have been observed are either linked to microsomal proliferation or of unknown significance, for instance in the lung (Narayan, Dani, and Misra, 1990). Some immunotoxic effects of DDT have been ascribed to inhibition of the functional activity of macrophages (Nunez, Estrada, and Calderon-Aranda, 2002).

Toxicity to humans

Acute intentional and accidental exposures

Early studies of the exposure of human volunteers to DDT focused on oral and dermal administration of large acute doses to volunteers particularly with respect to the storage of DDE and relative to animal studies. On the whole few effects were observed with perhaps paraesthesia of the tongue and mouth, confusion and slight tremors at high oral doses. Very high accidental or attempted suicidal exposure led to convulsions being observed in a few cases although the exact doses are unknown. Recovery was often within 1 or 2 days. In fact, the acute toxicity of DDT to humans seems remarkably low (Smith, 1991, 2001).

Chronic exposure and response

The safety record for humans in the use of DDT is phenomenally good considering the huge quantities distributed (Coulston, 1985; Smith, 1991, 2001). It was used for mass delousing in such a way that the bodies and inner clothing of thousands of people of all ages and states of health were liberally dusted. By necessity, the applicators worked in a cloud of the material. Other applicators have sprayed the interior of hundreds of millions of homes in tropical and subtropical countries under conditions of extensive dermal and respiratory exposure. Some people made or formulated DDT for many years. Dermatitis was commonly observed among workers who used DDT solutions but the rashes were probably due to the solvent. Paraesthesia of the extremities, headache, dizziness, and tremor of the tongue and hands have been linked with DDT production, but studies of other workers making or formulating DDT have shown little evidence of adverse effects to the individual or fertility (see Smith, 2001, for original references).

The most heavily exposed workers whose health have been investigated are those associated with malaria control. In Brazil, examinations failed to show consistent symptoms of DDT neurotoxicity although in Indian sprayers, serum DDT levels were 8.5 times higher than controls and visuomotor functions were slightly depressed (Misra, Nag, and Murti, 1984). The levels of DDT and its metabolites in the sera from applicators in malaria control in Natal were

significantly higher than in the population protected by the spraying. Although serum γ-gammaglutamyl transpeptidase was not statistically different from controls the mean in applicators was greater than the maximum laboratory mean level and alanine aminotransferase values were significantly greater in the applicators although not deemed clinically significant and possibly associated with alcohol consumption (Bouwman *et al.*, 1991a, 1991b). Members of households that had been sprayed inside with DDT had significantly greater levels in their serum than people from non-sprayed households. Recent findings from studies in Costa Rica have linked declines in neurobehavioural functioning and an increase in neuropsychological and psychiatric symptoms with DDT exposure of retired malarial sprayers, but no clinical differences were detected (Van Wendel de Joode *et al.*, 2001). A possible association between maternal blood DDE levels and pre-term and small birth weight for babies has been proposed recently (Longnecker *et al.*, 2001).

No significant overall cause of specific mortality excess among men potentially exposed at work to DDT from 1935 to 1976 was detected (Wong *et al.*, 1984) and similarly, a population living downstream from a defunct DDT manufacturing plant showed no DDT-specific illnesses or ill health, but possibly some changes in clinical chemistry, despite total DDT serum levels three times the national mean (Kreiss *et al.*, 1981). The induction by DDT level of microsomal enzymes of human liver was demonstrated first in workers and DDT may be more important than DDE in this regard (Poland *et al.*, 1970).

Evidence regarding mutagenic activity of DDT and its significance in humans is uncertain (Smith, 2001). Although there is a lot of evidence against DDT causing liver cancer in humans in Western countries, there is still the possibility of it acting as a promoter of potent carcinogens such as aflatoxin. Many studies have determined DDT levels in cases of cancer or other diseases but it is difficult to judge the significance of these (Cocco, Kazerouni, and Zahm, 2000; Smith, 2001; Snedeker, 2001; Turusov, Rakitsky, and Tomatis, 2002). A study of deaths that occurred among men who used DDT in an antimalarial campaign in Sardinia in the late 1940s showed that workers had a significant increased risk for liver and biliary tract cancers and multiple myeloma. However, non-exposed workers also showed elevated incidences of cancer (Cocco *et al.*, 1997).

There have been reports that pancreatic cancer might be associated with exposure to DDT and ethylan in a nested case–control mortality study among workers at a chemical plant followed by interviews with next of kin and co-workers and examination of work records (Garabrant, Held, and Homa, 1993; Garabrant *et al.*, 1992; Malats, Real, and Porta, 1993). However, this has not been confirmed in wider non-occupational surveys (Cocco, Kazerouni, and Zahm, 2000).

Plasma and tissue levels of DDT have been particularly targeted as being linked with a rising incidence of breast cancer (Dewailly, Ayotte, and Dodin, 1997; Dewailly *et al.*, 1994a, 1994b; Wolff *et al.*, 1993). Much of the evidence has been reviewed in detail and is not supportive or is inconclusive (Ahlborg *et al.*,

1995; Cocco, Kazerouni, and Zahm, 2000; Key and Reeves, 1994; Snedeker, 2001; Wolff *et al.*, 2000a, 2000b). Elevated levels of DDT or DDE were reported in cancerous breast tissue fat compared with tissue from benign mammary disease (Guttes *et al.*, 1998). In contrast, a number of studies have found no relationship between blood levels of DDE and risk of, or progression of, breast cancer (Hunter *et al.*, 1997; Schecter *et al.*, 1997; Snedeker, 2001). The study of Schecter *et al.* (1997) is of particular interest in that women in North Vietnam were examined who had generally high levels of DDT or DDE due to exposure from antimalarial use.

Levels in fat, blood, and milk

The highest reported storage of DDT and related compounds was that of a healthy worker whose fat contained DDT and DDE at concentrations of 648 and 483 ppm respectively, but most have been considerably lower (Smith, 1991, 2001). An important point is that it has always been difficult to assess exposure. Considerable evidence suggests that with time the greatest proportion of DDT in people is as the metabolite DDE (Smith, 1991). Although each person without special exposure to DDT has relatively constant serum levels of DDT and DDE, DDE values differ more than the DDT values from person to person. Levels of DDT and its metabolites in the serum of adults rose over a 12-month period following application of the pesticide to their homes in KwaZulu, South Africa. In contrast, levels fell in the age group 3–20 years, showing the complexity of any pharmacokinetic interpretations (Bouwman, Becker, and Schutte, 1994). Surveys have demonstrated a gradual decline in the concentrations of DDT and related compounds in human fat (Smith, 1991; Snedeker, 2001; Turusov, Rakitsky, and Tomatis, 2002). Presumably a similar decline has occurred in the levels of these compounds in human serum. Consumption of fish appeared to be a predictor of plasma DDE levels but most reliable were age and serum cholesterol (Laden *et al.*, 1999).

No information is available on the secretion of DDT in the milk of women who were occupationally exposed to the compound or who were made ill by it, regardless of circumstances. As with other chlorinated pollutants, women may be in negative DDT balance during lactation and this may be a significant factor in determining the lower levels of DDT found in women than men in the general population. In areas of KwaZulu, human milk levels of DDT and metabolites were significantly higher in women whose houses had been treated with DDT to interrupt malaria transmission. (Bouwman *et al.*, 1990a, 1990b). Primiparous mothers had significantly more than multiparous mothers and transfer from the mother's milk to the child's blood was clearly demonstrated (Bouwman *et al.*, 1992). Overall, despite the presence of very low levels of DDT in human milk and placenta, the risk to neonates is judged to be low although caution is still warranted (Hardell, Lindstrom, and Van Bavel, 2002). Clearly, levels would have to be very high before any advice against breast-feeding could be given.

Ethylan

Ethylan is the p,p'-ethyl analogue of DDT (Figure 2.4) with a trade name of Perthane that has been used sometimes. It was introduced in 1950 to control pear psylla, leaf hoppers, larvae on vegetables and moths, and carpet beetles in textiles.

Toxicity to laboratory animals

The oral toxicity of ethylan seems to be very low at >4 g/kg in rats and mice (Gaines, 1969) although not surprisingly the intravenous toxicity is much higher (73 and 173 mg/kg, respectively). Minimal liver changes of rats are observed at doses of 2–5 g/kg of diet for prolonged periods (Smith, 2001). Although rats do store ethylan in fat as with other chemicals of this type, it is reasonably well metabolized. Like o,p'-DDD, ethylan administered to dogs affects corticosteroid excretion and causes marked atrophy of the adrenal cortex. This is not seen in rats. The evidence that ethylan can induce tumours in mice is very weak and non-existent for rats (Smith, 2001).

Toxicity to humans

As a potential anti-cancer agent, ethylan was administered to men with prostatic carcinoma and women with carcinoma of the breast at up to 200–300 mg/kg for 6 days. Smaller doses caused diarrhoea, vomiting, and nausea in some patients but there were signs that patients began to tolerate higher doses. Marked depression of plasma 17-hydroxycorticosteroid levels was observed although still within the normal range (Taliaferro and Leone, 1957). An association between exposure to ethylan and development of pancreatic cancer has been reported (see DDT) but there is a degree of uncertainty as exposure was based on self-reporting of the general population (Garabrant *et al.*, 1992).

Methoxychlor

Methoxychlor is 1,1,1-trichloro-2,2,-bis(4-methoxyphenyl) ethane (Figure 2.4) and is thus the p,p'-dimethoxy analog of DDT. It was effective against many insects affecting fruits, vegetables, and livestock. The acute oral toxicity of methoxychlor to rodents is much lower than other organochlorine insecticides (Table 2.1), probably due to its rapid metbolism (Cabral *et al.*, 1979; Smith, 2001). Thus the attractiveness of methoxychlor for use was its relatively short biological half-life and low mammalian toxicity.

Absorption, distribution, metabolism, and excretion

Following oral administration of radioactive methoxychlor to mice, 93 per cent was recovered from the excreta within 24 h including 2-(p-hydroxyphenyl)-1,1-trichloro-

ethane, 2,2-*bis*(*p*-hydroxy-phenyl)-2′-(methoxyphenyl)-1,1,1-trichloroethane and 2,2-*bis*(*p*-hydroxyphenyl)-1,1-trichloroethane, which were eliminated largely in conjugated form (Kapoor, 1970). Since then there has been a great many studies on its metabolism using liver microsomes, purified cytochrome P-450 isoforms, or insect cells containing human cytochrome P-450 species to form both the mono- and dihydroxy products as well as more polar compounds (see Figure 2.6 for a simplified view). In the rat, demethylation seems to occur with CYP2B isoforms with some evidence for additional involvement of CYP2C6. In humans, CYP2C19 and CYP1A2 seem to be responsible for demethylation. *Ortho* hydroxylation of the monodemethylated products is predominantly catalysed by CYP3A4. On the whole, bidemethylation and *ortho* hydroxylation seem to occur less readily with human samples than with rat liver (Kishimoto and Kurihara, 1996; Kupfer and Bulger, 1987; Smith, 2001; Stresser and Kupfer, 1997, 1998a, 1998b). Phenolic products appear to be converted to activated intermediates, which may bind covalently to macromolecules (Bulger and Kupfer, 1990; Kupfer, Bulger, and Theoharides, 1990). Microsomal metabolism of methoxychlor in rat liver has been reported to result in binding to iodothyronine 5′-monodeiodinase with resulting depression of

Figure 2.6 Simplified scheme of methoxychlor metabolism

iodinase activity *in vivo* but the significance of these findings on the thyroid hormone metabolism and action is unknown (Zhou *et al.*, 1995). In rats, methoxychlor induced hepatic CYP2B1 and CYP3A enzymes levels, but in regimes with multiple treatment and less efficiently than DDT (Li, Dehal, and Kupfer, 1995).

Effects on organs and tissues

Mixed mutagenicity results have been reported for methoxychlor (Oberly *et al.*, 1993) and increased occurrence of neoplasms of all sorts have been shown in rats and carcinoma of the testes in some mice only after feeding high doses of the technical product for 2 years (Reuber, 1979a, 1979b). Large doses of methoxychlor also produce dosage-dependent chronic nephritis and hypertrophy of the kidneys, mammary glands, and uteri of swine and cystic tubular nephropathy in rats (Tegeris *et al.*, 1966; Tullner, 1962). The liver seems relatively insensitive to methoxychlor but testicular changes have been frequently observed as well as atresia of ovarian follicles, albeit at high doses (Bal, 1984; Smith, 2001; Tullner, 1962).

Effects on reproduction

Large doses of methoxychlor have oestrogenic effects and mating and litter size of rats and mice are reduced, male and female offspring reproductive performance is decreased, and female pups may have early vaginal opening (Cummings, 1997). The potency appears to be 1/10 000th of diethylstilboestrol.

The oestrogenicity of methoxyclor probably is due to metabolites not the parent compound. *In vitro* pure methoxychlor itself is not oestrogenic whereas metabolites are, for instance *bis*(hydroxyphenyl)trichloroethane (Bulger, Muccitelli, and Kupfer, 1978a, 1978b). The oestrogenic activity *in vivo* of impure methoxychlor in inducing paramers of uterine proliferation, uterus growth, uterine orinithine decarboxylase and epidermal growth factor receptor, creatine kinase and peroxidase may be partly caused by the demethylated analogues as metabolites and impurities that are also metabolites (Bulger, Feil, and Kupfer, 1985; Bulger, Muccitelli, and Kupfer, 1978a; Cummings, 1997; Cummings and Metcalf, 1994; Kupfer and Bulger, 1987; Metcalf, Laws, and Cummings, 1996). By both *in vitro* and *in vivo* criteria, 1,1-dichloro *bis*(4-hydroxyphenyl)ethane is the most potent agent. Methoxychlor affects the decidual cell response of the rat uterus and embryo transport rate might also be effected (Cummings, 1997; Cummings and Gray, 1987; Cummings and Perreault, 1990).

Some evidence suggests that reproductive effects of methoxychlor metabolites in male rats (Bal, 1984; Tullner, 1962) may be mediated, in part, by elevation of prolactin concentration and release, which in turn influences hypothalamic levels of gonadotropin-releasing hormone (Goldman *et al.*, 1986). It has been reported that methoxychlor fed to lactating dams affected the reproductive tract of suckling females (Appel and Eroschenko, 1992). In studies of the effect of methoxychlor on

reproductive tract development following neonatal exposure of mice, precocious vaginal opening, cornification and increased tract size, and ovarian atrophy were observed in females and reduced serum testosterone, testicular DNA content, seminal vesicles, and prostate in males (Cooke and Eroschenko, 1990; Eroschenko, 1991; Eroschenko, Abuel-Atta, and Grober, 1995; Eroschenko and Osman, 1986). Rats dosed with methoxychlor before and following birth had immune and reproductive changes at doses of 0, 5, 50, or 150 mg kg^{-1} day^{-1} in a large study. Primary adult effects were reproductive and 5 mg kg^{-1} day^{-1} was not a NOAEL (Chapin *et al.*, 1997). Methoxychlor prevented ovariectomy-induced bone loss in the rat (Dodge *et al.*, 1996).

The oestrogenic effects of methoxychlor are not restricted to those on uterine or other reproductive physiology and function. Both running wheel activity (oestrogen controlled) and sex behaviour in rats and hamsters were induced by 400 mg kg^{-1} day^{-1} (Gray *et al.*, 1988). Exposure of pregnant mice to methoxychlor seems to cause changes in behaviour of male offspring (vom Saal *et al.*, 1995). Although it is usually assumed that the methoxychlor metabolites act in an identical manner to oestradiol, this may not always be true (Eroshenko, 1991; Cummings, 1997).

Toxicity to humans

The rapid metabolism and excretion of methoxychlor suggests that at most exposure levels humans are at little risk, including absorption from cow's milk. Volunteers given methoxychlor at rates of up to 2 mg kg^{-1} day^{-1} for 8 weeks were without detectable effect on health, clinical chemistry, or the morphology of blood, bone marrow, liver, small intestine, or testis; the highest dosage administered being similar to that considered safe for occupational intake at the time (Stein, Serrone, and Coulston, 1965). Apparently there has been no confirmed case of poisoning, occupational or otherwise, firmly attributed to methoxychlor and residues have rarely been found in human tissues (Smith, 2001).

Mirex and chlordecone

Structurally, mirex (1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1,3,4-metheno-1*H*-cyclobuta[*c*,*d*]-pentalene) and chlordecone (1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-1,3,4,-metheno-2*H*-cyclobut[*c*,*d*]pentalen-2-one) resemble the chlorinated cyclodiene insecticides but with an interesting cage structure (Figure 2.7). They are only slowly metabolized often concentrating many thousand-fold in food chains. However, unlike the cyclodienes they do not immediately cause sudden seizures. Thus, in some respects in action they more resemble DDT and its analogues. Mirex was a stomach insecticide for consumption by fire ants whereas chlordecone was used as a contact insecticide against leaf cutting insects and fly

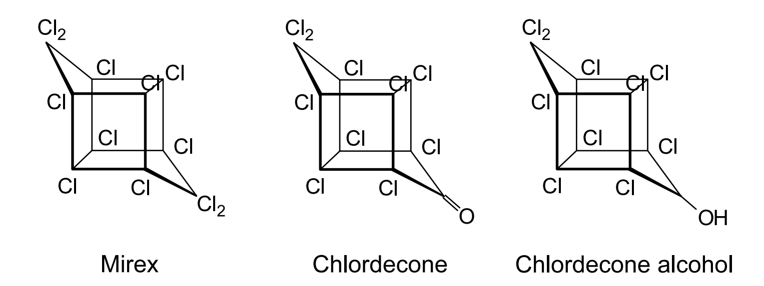


Figure 2.7 Structures of mirex, chlordecone, and its metabolite chlordecone alcohol (chlordecol)

larvae. In many ways these two insecticides have properties in common, but in other aspects have different metabolism and toxicities dependent on the ketone function in chlordecone.

Mirex

Mirex is virtually insoluble in water and, not surprisingly, mirex is poorly absorbed from the gastrointestinal tract and slowly metabolized. A half-life in the rat is about 100 days (Mehendale *et al.*, 1972). The major dechlorination products in the rat are 2,8-dihydromirex and 5,10-dihydromirex. The latter appears to be converted to more polar metabolites that can be excreted in the urine (Yarbrough *et al.*, 1983).

Experimental in vivo *toxicity*

The acute oral toxicity of mirex to rats ranges from approximately 300 to 3000 mg/kg (LD_{50}) and 250 and 125 mg/kg for male and female hamsters, respectively (Cabral *et al.*, 1979; Gaines and Kimbrough, 1970; Smith, 1991). Rats fed diets containing 1280 ppm for up to 13 weeks showed hyperexcitibility, decreased haemoglobin levels, tremors, and even convulsions before death occurred (Larson *et al.*, 1979a). Concern about the possibility of photomirex (8-monohydromirex) formed in the environment being more toxic than the parent compound is still not satisfactorily resolved. Enlarged livers have been observed in rats, mice, and rabbits with dietary levels of between 5 and 80 ppm but not in dogs at 20 ppm (Larson *et al.*, 1979a; Pittz *et al.*, 1979; Warren, Kirkpatrick, and Young, 1978; Yarbrough *et al.*, 1981). Some investigations have found persistence of effects on the liver one year following dosage (Chu *et al.*, 1981) and there seems good evidence for mirex causing increased biliary tree dysfunction and impaired bile acid secretion and probably reflects changes in ATPase transport pumps at the cell membranes (Curtis and Hoyt, 1984; Curtis and Mehendale, 1981; Mehendale, 1977; Teo and Vore, 1991). As one might expect, the proliferation of the hepatic endoplasmic reticulum

is associated with induction of cytochrome P-450 isoforms. Even at 1 ppm in the diet mirex can induce cytochrome P-450 enzyme mediated activities in both rats and mice (see Smith, 1991) but it is difficult now to relate these to current knowledge of specific isoforms that might be involved. Mirex and chlordecone might act in a similar way to induce CYP isoforms but there are distinct differences between these chemicals (Crouch and Ebel, 1987; Ebel, 1984).

Long-term administration of mirex in the diet to rats and mice causes hepatic adenomas and carcinomas probably by an epigenetic mechanism of uncertain mechanism (Abraham, Benitz, and Mankes, 1983; Innes *et al.*, 1969; Ulland *et al.*, 1977). Mirex can act as a promoter of skin carcinogenesis but apparently of a different cell population that is activated by phorbol esters (Kim *et al.*, 1997; Meyer *et al.*, 1993; Moser, Meyer, and Smart, 1992; Moser, Robinette, and Smart, 1993). The significance of these findings for human health is questionable and a number of *in vitro* mutagenicity tests have proven negative (Smith, 1991). Possibly related to the liver effects is the depression of serum triodothyronine and thyroxine levels with reduction of colloid density in the thyroid (Singh *et al.*, 1985; Yarbrough *et al.*, 1981).

Chronic administration of low doses of mirex (e.g. 5 ppm in the diet to mice) seems to cause reduced litter size and viability in rats and mice (Gaines and Kimbrough, 1970) and offspring of rat mothers exposed to mirex may develop cataracts (Rogers and Grabowski, 1984) but this seems to be an indirect effect perhaps associated with oedema or protein insufficiency. Both mirex and photomirex damage rat testes causing hypocellularity of seminiferous tubules and decreased spermatogenesis (Yarbrough *et al.*, 1981).

Chlordecone

Chlordecone is the 2,2 dechlorinated 2-oxo analogue of mirex (often known by its trade name Kepone) and is slightly more soluble in water than mirex.

Absorption and metabolism

The greater aqueous solubility of chlordecone means that, unlike mirex, it is more easily absorbed from the intestine and is probably more widely distributed to the blood and tissues other than fat. Compared with some other polyhalogenated pesticides and related chemicals, the decline of chlordecone from tissues is relatively rapid and a significant proportion of a single dose can be excreted in the milk of lactating rats (Kavlock *et al.*, 1980). Normally, in both rats and humans, a major route of excretion is in the faeces, in humans some occurring by secretion of the pesticide through the intestinal wall (Boylan, Egle, and Guzelian, 1978; Egle *et al.*, 1978). Chlordecone induces a number of cytochrome P-450-mediated oxidations in the male rat liver, as might be expected (Chambers and Trevathan, 1983). The presence of the ketone function in chlordecone provides a simple route for

metabolism by reduction of the ketone group to give chlordecone alcohol (chlordecol) and subsequent glucuronidation (Blanke *et al.*, 1978; Fariss *et al.*, 1980). Interestingly, this is only a minor metabolite in rats, mice, and guinea pigs but much more significant in humans and the Mongolian gerbil (Houston *et al.*, 1981). The species differences seemed to be explained by the presence of a specific aldo-ketose reductase in humans, gerbils (Molowa, Shayne, and Guzelian 1986a; Molowa *et al.*, 1986b) and possibly pigs (Soine, Blanke, and Schwartz, 1983). Despite the formation of chlordecone alcohol in humans, the overall rate of clearance of chlordecone appears to be greater in rats and mice.

Experimental in vivo *toxicity*

Deaths of rats and mice from chlordecone are preceded by abnormal gait and severe tremors. The acute dermal LD_{50} values for rats are approximately 10 times that of the oral doses, reflecting the solubility of the pesticide in the intestinal tract (Egle, Guzelian, and Borzelleca, 1979; Gaines, 1969; Larson *et al.*, 1979b; Smith, 1991). Chronic administration leads to a decreased weight gain, liver enlargement as well as changes in the adrenals (hyperplasia of the zona fasiculata and zona reticularis), kidney, and testes (Baggett, Thureson-Klein, and Klein, 1980; Klingensmith and Mehendale, 1982). Over a prolonged period hepatocellular carcinomas and adenomas occur to an increased degree in mice and rats that survive chronic administration of chlordecone at low doses in their diets (approximately 20 ppm) for up to 2 years (Larson *et al.*, 1979b; NCI, 1976; Reuber, 1978). There is little evidence to support the view that chlordecone is a mutagen in its hepatocarcinogenic action (Smith, 1991) and is more likely acting as an epigenetic carcinogen (Sirica *et al.*, 1989). The most characteristic feature of chlordecone toxicity is the effects on reproduction described below.

Mechanistic studies

A great many studies have been concerned with the inhibition of mitochondrial and membrane bound Na^+, K^+-ATPases, and certain Mg^+-ATPases caused by chlordecone perhaps associated with energy production (see Smith, 1991). Other studies on both liver and brain implicate aspects of calcium metabolism linked to ATPases (Herr, Gallus, and Tilson, 1987; Hoskins and Ho, 1982) in a mechanism that does not occur with mirex. It was proposed that chlordecone increases free intrasynaptosomal Ca^{2+} by a non-specific leakage through the plasma membrane and by passage through voltage-sensitive Ca^{2+} due to membrane depolarization (Komulainen and Bondy, 1987). This is compatible with increased synaptosomal calcium levels causing depolarization of the membrane-enhancing release of neurotransmitters. Chlordecone may, through activation of serotonin neurons, cause decreased GABAergic activity in the striatum and thereby an increase in cholinergic tone, inducing tremors (Gandolfi *et al.*, 1984). The relationship between brain

ATPases, calcium homeostasis and neurotransmitter turnover is still not understood. It is known that chlordecone does not bind to the picrotoxin binding site of the GABA–receptor complex as do lindane, cyclodienes, and toxaphenes (Lawrence and Casida, 1984).

A variety of studies have demonstrated that chlordecone potentiates CCl_4 hepatic toxicity and that partial hepatectomy protects against this. Potentiation occurs at doses of chlordecone that do not increase lipid peroxidation or cytochrome P-450 activity (Mehendale, 1989; Smith, 1991). The findings do not seem to be explained by greater CCl_4 metabolism. Chlordecone enhances the suppression of calcium sequestration by microsomes (Hegarty, Glende, and Recknagel, 1986) and it is thought that chlordecone suppresses hepatocellular regeneration after necrosis caused by CCl_4 (Carpenter *et al.*, 1996; Chaudhury and Mehendale, 1991; Kodavanti, Rao, and Mehendale, 1993; Kodavanti *et al.*, 1992; Rochelle and Curtis, 1994; Soni and Mehendale, 1993; Soni and Mehendale, 1998) although neonatal rats are resistant to this effect (Cai and Mehendale, 1993; Dalu and Mehendale, 1996; Dalu, Rao, and Mehendale 1998; Dalu *et al.*, 1995; Gilroy, Carpenter, and Curtis, 1994). Chlordecone also seems to inhibit the transfer of metabolites from hepatocyte to the bile canaliculus possibly by interference of particular ABC transporters (Gilroy, Carpenter, and Curtis, 1994; Guzelian, 1982).

Reproductive effects

Early studies in rats, mice, and birds showed clearly that chlordecone affects breeding performance. Chronic or multiple doses of parents had a significant effect on numbers and size of offspring (Guzelian, 1982; Smith, 1991). Surviving rat fetuses exhibited reduced body weight, reduced ossification, oedema, undescended testicles, enlarged renal pelves, and enlarged cerebral ventricles (Chernoff and Rogers, 1976). Some of the reproductive effects are probably due to chlordecone acting as an oestrogen (Smith, 1991). In uterotrophic assays on neonatal mice, chlordecone has marked dose-related effects on both the vagina and uterus (Eroschenko and Osman, 1986; Uphouse and Eckols, 1986). However, as we would now expect from present knowledge of the action of oestrogens and xeno-oestrogens with the oestrogen α and β receptors, the action of chlordecone was not identical to that of oestradiol. In some experiments chlordecone caused decreases in spermatozoa motility and viability (Linder *et al.*, 1983) but does not seem to produce dominant lethal effects (Simon *et al.*, 1986).

Human toxicity

An important toxicological incident occurred when workers manufacturing chlordecone under poor safety control were chronically exposed to the pesticide (Guzelian, 1982, 1992). Of 133 employees or former employees examined 57 per cent had symptoms of poisoning. In those most affected, this was manifest as weight loss,

headache, tremor, especially of the upper extremities, muscle weakness, unusual eye movements, ataxia, slurred speech, skin rash, and abnormalities of normal liver function (Taylor *et al.*, 1978). It was concluded that absorption probably occurred by the dermal route and some symptoms persisted in some patients for at least 2 years (Taylor, 1985). Pathological examination of the sural nerve, muscle and liver biopsies showed a variety of changes including accumulation of elongated, electron dense membranous bodies in Schwann cells, electron-dense structures below the sarcolemma and between myofibrils, and proliferation of the hepatic endoplasmic reticulum (Guzelian, 1982; Smith, 1991). Of 28 chlordecone-poisoned workers examined, only 8 patients had normal sperm counts and in only one was chlordecone blood level above 1 ppm (Guzelian, 1982). Arrest of sperm maturation was observed in testicular biopsies from some patients. Neurotoxicity was associated with blood levels of chlordecone $>0.1-1$ ppm. Chlordecone was found not just in the blood of affected workers at the plant but in non-affected workers, relatives, workers in nearby businesses, and even the general community (Cannon *et al.*, 1978). Plasma levels relative to adipose tissue seemed to be much higher than expected with some other chlorinated insecticides and may reflect specific binding to albumin and lipoproteins (Soine *et al.*, 1982). Most of excreted chlordecone in humans is in the faeces and some as the glucuronide of chlordecol (Fariss *et al.*, 1980). Subsequent work showed that expression of the aldo-keto reductase responsible for chlordecol formation varied considerably between individuals (Molowa, Shayne, and Guzelian, 1986a; Molowa *et al.*, 1986b). This episode has been presented as an illustration of some of the limitations of animal experiments in predicting human toxicity (Guzelian, 1992).

Regulatory aspects

In the European Union and the North American Free Trade Area organochlorine insecticides are nowadays rarely used in agriculture. Lindane, the last organochlorine to be used in food production in the European Union, was revoked in 2001. Nevertheless, lindane retains a temporary acceptable daily intake of 0.001 mg/kg body weight assigned by the Joint Expert Meeting on Pesticides Residues in 1997 (FAO/WHO, 1998). The remaining uses of lindane in the United States for targeting lice and treating scabies as well as some other uses, are also under pressure (Weinhold, 2001). It is many years since DDT was used in food production, but because of its persistence in the soil, residues of DDT and its metabolites continue to occur in commodities. To facilitate international trade, the Joint Expert Meeting on Pesticides Residues assigned DDT a provisional tolerable daily intake in 2000 (FAO/WHO, 2001). The complex arguments concerning a total worldwide ban in the light of the problems in combating malaria have been well discussed (Attaran and Maharaj, 2000; Tren, 2001). (See also http://www.malaria.org/DDTpage.html)

Summary

The organochlorine insectides no longer have widespread, worldwide use. However, it would be unwise to dismiss their toxicology as now of little interest. First, they have been of immense use in the general studies of the mechanisms of neurotoxic agents, for instance the role of different GABA receptors, and in the current concerns of environmental agents that may have hormone disrupting properties. Secondly, as a result of indiscriminate agricultural use decades ago residues of these chemicals will persist in the biomass for many years to come and hence concerns for the implication for human health will continue. Thirdly, as the recent debate on DDT has reminded us, perhaps there may be a role for the use of some of these highly effective insecticides in the future, under strictly controlled conditions, in circumstances of world health we have not yet envisaged.

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3 Anticholinesterase Insecticides

Charles M. Thompson and **Rudy J. Richardson**

Introduction

The principal mechanism for the mammalian toxicity of anticholinesterase insecticides is inhibition of the intended target, acetylcholinesterase (AChE), in the peripheral and central nervous systems (PNS and CNS, respectively) (Fukuto, 1990; Mileson *et al*., 1998). Unintended targets whose interactions with these insecticides have been well characterized both *in vitro* and *in vivo* include butyrylcholinesterase (BChE) and neuropathy target esterase (neurotoxic esterase, NTE). Although BChE may serve as a sensitive biomarker of exposure, its inhibition is not a basis of toxicity (Chen *et al*., 1999). Indeed, mutations leading to diminished function or complete absence of BChE have no apparent adverse effect (Massoulié *et al*., 1993). Inhibition and ageing of a critical level of neural NTE can initiate organophosphorus compound-induced delayed neurotoxicity (OPIDN) (Richardson, 1998). Nevertheless, anti-AChE insecticides in current use cannot inhibit NTE to a sufficient degree at sublethal doses to pose a neuropathic risk (Mileson *et al*., 1998; Moretto and Lotti, 1998; Richardson, 1992). Finally, recent work using AChE knockout mice supports the idea that other non-AChE targets contribute to the toxicity of anti-AChE compounds (Duysen *et al*., 2001; Xie *et al*., 2000). These non-AChE targets and their relationship to toxicity, however, require further elucidation to be useful in risk assessment (Mileson *et al*., 1998; Pope, 1999). Accordingly, this chapter concentrates on the following topics: characteristics of AChE, classes of anti-AChE insecticides, toxicological consequences of AChE inhibition, therapies for the cholinergic toxicity resulting from AChE inhibition, and regulatory aspects of anti-AChE insecticides.

AChE

Classification and physiological function

AChE has been isolated from a variety of animal species (Fukuto, 1990; Sultatos, 1994). This enzyme belongs to a large family of serine hydrolases, separate from the serine proteases, that contain a common structural motif, the α/β hydrolase fold (Ollis *et al.*, 1992). In addition to AChE (Soreq *et al.*, 1990), this protein family includes BChE (Lockridge *et al.*, 1987), cholesterol esterase (Kyger, Wiegand, and Lange, 1989), and various lipases (Derewenda, 1994) and carboxylesterases (Korza and Ozols, 1988; Long *et al.*, 1988). NTE is a novel serine hydrolase that does not belong to the α/β hydrolase fold superfamily or any other known category of serine hydrolases (Glynn, 1999).

The cholinesterases (collectively AChEs and BChEs from various species and tissues) are remarkable enzymes (Broomfield *et al.*, 1995) within the family of α/β hydrolase fold proteins. By virtue of their active site characteristics, they are particularly efficient at catalysing the hydrolytic breakdown of esters that bear quaternary ammonium groups ($—NR_3^+$). Indeed, the specific biological role of AChE is the rapid termination of the neuronal impulse that occurs when acetylcholine (ACh) is released into the synaptic cleft. As an important means of regulating the depolarization of post-synaptic cells, AChE removes ACh from the subsynaptic space by accelerating its hydrolysis to acetate (CH_3COO^-) and choline ($HOCH_2CH_2N(CH_3)_3^+$). AChE is an exceptionally efficient enzyme with one of the highest turnover numbers known for its natural substrate ($>10^4\,s^{-1}$) (Quinn, 1987; Rosenberry, 1975; Taylor and Radić, 1994). Although AChE can hydrolyse a variety of esters, its active site appears to be optimized for ACh and its sulphur or selenium analogues. Choline esters bearing an acyl group of butyryl or larger are hydrolysed very slowly. BChE, in contrast, hydrolyses these larger substrates with ease, although the turnover numbers for BChE pale in comparison with those for AChE.

Within a particular mammalian species, AChE is a single enzyme that is encoded by a single gene distinct from the genetic encoding for BChE (Chatonnet and Lockridge, 1989; Massoulié *et al.*, 1999). Nevertheless, polymorphisms of AChE exist with respect to quaternary structure, mode of attachment to membranes, and post-translational maturation. These molecular forms correspond to distinct tissue expression and arise from the process of alternative splicing (Li *et al.*, 1991; Taylor *et al.*, 1993). Invariant exons of the AChE gene coding for the constant and major portion of the enzyme, the catalytic domain, are spliced with alternative exons coding for the variable and minor C-terminal region of the molecule (Massoulié *et al.*, 1999). Thus, molecular forms of the enzyme containing different numbers of catalytic subunits are found in the CNS and PNS as well as in non-neural loci, including erythrocyte membranes (Lawson and Barr, 1987) and plasma (Chen *et al.*, 1999) of some species. The physiological function of AChE occurring outside

of synapses or myoneural junctions is unknown, but for an individual species the catalytic domains of such forms are the same as those in brain, muscle, or other tissues (Aziz-Aloya, Sternfeld, and Soreq, 1993; Heider *et al.*, 1991).

Some differences among molecular forms of AChE may also arise from post-translational modifications. For example, brain and erythrocyte AChEs are differentially glycosylated (Liao *et al.*, 1992) and can be distinguished immunochemically (Boschetti *et al.*, 1996; Novales-Li and Priddle, 1995; Rakonczay and Brimijoin, 1985). Nevertheless, these differences do not appear to affect substantially any kinetic parameters (pharmacodynamics) of the enzyme, including its intrinsic sensitivity to inhibitors (Velan *et al.*, 1993). Apparent disparities in pharmacodynamic properties between AChEs from different tissues from the same species disappear if the enzyme is isolated from potentially interfering extrinsic factors, e.g. by immunoprecipitation (Mortensen *et al.*, 1998). Thus, even though different polymorphic forms of AChE are found in brain, erythrocytes, and peripheral tissues of a given species, the catalytic domains contained in these molecular forms have identical primary sequences and appear to have indistinguishable pharmacodynamic properties (Aziz-Aloya, Sternfeld, and Soreq, 1993; Mendelson *et al.*, 1998).

Knowledge of the structural and pharmacodynamic similarities between brain and erythrocyte AChE within a given species has provided a rational basis for using erythrocyte AChE inhibition by anti-AChE insecticides as a surrogate measure of brain AChE inhibition by these compounds (Chen *et al.*, 1999; Lotti, 1995).

Similarly, it is known that BChE found in plasma or tissues is distinct from AChE, potently inhibited by anti-AChE compounds, and may be genetically deficient or absent without apparent ill effect (Chatonnet and Lockridge, 1989; Massoulié *et al.*, 1993). These observations have led to the conclusion that BChE inhibition is not a suitable endpoint for assessing toxicity, but may be used as a biomarker of exposure to anti-AChE insecticides (Chen *et al.*, 1999).

Structure

The three-dimensional structure of AChE derived from X-ray crystallography or nuclear magnetic resonance reveals three functional regions of the enzyme: (a) a catalytic triad that operates the active site mechanics, (b) a gorge that connects the active site region to the protein surface, and (c) a peripheral anionic site at the surface (Bourne, Taylor, and Marchot, 1995; Bourne *et al.*, 1999; Sussman *et al.*, 1991). A tube diagram that depicts the α-helical and β-sheet motifs of AChE with the substrate nestled in the active site is shown in Figure 3.1.

The active site residues of the catalytic triad (Ser200, His440, and Glu327) of AChE reside at the base of the gorge. Although a high degree of sequence homology exists for AChEs across a variety of species, there are some variations in alignment (Massoulié *et al.*, 1993). Therefore, the numbers given for the catalytic triad and other residues of the protein refer to the sequence of AChE from *Torpedo californica* (Sussman *et al.*, 1991), which is often used as a standard. The catalytic

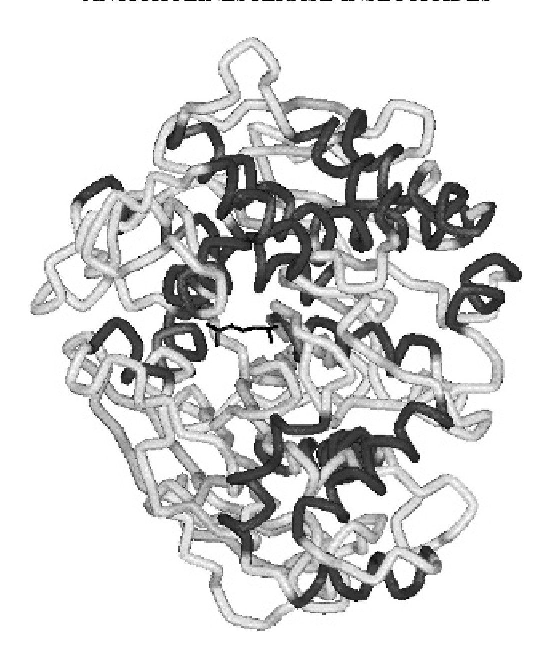


Figure 3.1 Tube diagram of backbone structure of AChE showing regions containing α-helices (dark grey) and β-sheets (light grey). A stick structure of the substrate, ACh (black), is shown in the active site

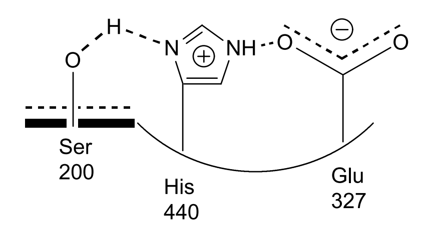


Figure 3.2 Catalytic triad (Ser200, His440, Glu327) of AChE showing hydrogen-bond activation of active site serine

triad of AChE (Figure 3.2) is functionally similar to that of the serine proteases, e.g. trypsin and chymotrypsin, but it differs in certain respects. For example, its acidic residue is Glu rather than Asp, its primary sequence order is reversed (Ser, His, Glu versus Asp, His, Ser), and its three-dimensional structure is essentially a mirror image of the serine protease catalytic triad (Ollis *et al.*, 1992; Sussman *et al.*, 1991). Moreover, the details of how the hydroxyl group of the active site Ser is rendered

nucleophilic appear to differ somewhat between the serine proteases and AChE. In the serine proteases, the catalytic mechanism involves a multiple proton-transfer or charge-relay system involving the Asp carboxyl, His imidazolium, and Ser hydroxyl (Massoulié *et al.*, 1993). In AChE, there may be transfer of only a single proton (Quinn, 1987; Taylor and Radić, 1994) and/or the formation of short, strong hydrogen bonds between the active site His and at least one of two active site Glu residues, one of them being in the catalytic triad (Massiah *et al.*, 2001).

A molecular model of the catalytic triad and other important residues in the active site of AChE is shown in Figure 3.3. The figure illustrates the addition of the serine hydroxyl to the carbonyl group of ACh to form the acyl intermediate. The His440 residue is conserved in all members of the serine hydrolases in the α/β hydrolase fold and serves as a reference position. Adjacent to the active site cluster is an oxyanion hole formed by main-chain N—H groups contributed by Gly118, Gly119, and Ala201 (not shown). These hydrogen bond donors stabilize complexes of the enzyme with the transition state of its substrate as well as with covalent and transition state analogue inhibitors (Ordentlich *et al.*, 1998). Aromatic side-chain residues (Phe288 and Phe290) form the acyl pocket responsible for the acetyl ester specificity of AChE. Trp84 is part of the choline-stabilizing subsite (along with Tyr330 and Glu199, not shown) (Harel *et al.*, 1995, 1996; Taylor and Radić, 1994).

The gorge region of AChE is approximately 20 Å in depth and is lined with 14 aromatic residues (Axelsen *et al.*, 1994; Koellner *et al.*, 2000). The substrate, ACh, must move through the entire depth of the gorge to access the catalytic triad in the active site. The lipophilic lining causes a reduction in the interaction with cationic

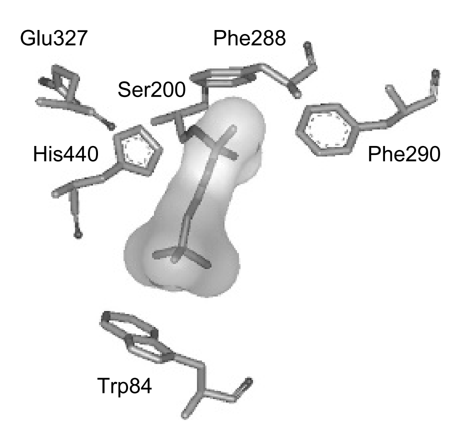


Figure 3.3 Molecular model depicting addition of the serine hydroxyl to the carboxylester group of ACh to form the acyloxy intermediate. Amino acid residues shown include the catalytic triad (Ser200, His440, Glu327), acyl pocket (Phe288, Phe290), and part of the choline-stabilizing subsite (Trp84). The backbone of ACh is shown through a transparent hydration sphere

substances, e.g. ACh, allowing them to traverse the gorge readily. Because conformationally extended ACh is larger than the narrowest part of the gorge, dynamic spatial accommodations in the gorge wall are needed to allow the substrate to traverse the 20 Å distance to the active site (Wlodek *et al.*, 1997; Wlodek, Shen, and McCammon, 2000). A point of constriction, formed principally by the side chains of Tyr121 and Phe330, separates the upper and lower parts of the gorge (Koellner *et al.*, 2000).

Before elucidation of the three-dimensional structure of AChE, binding of ACh was thought to be stabilized by a discrete anionic site near the active site serine estimated to consist of as many as 6–9 formal charges. Current information indicates that the facilitated binding of ACh occurs by a combination of processes. These include aromatic guidance of ACh through the gorge, stabilization by the choline-binding subsite, and longer-range Coulombic forces (Massoulié *et al.*, 1993; Sussman *et al.*, 1991; Taylor and Radić, 1994).

The peripheral anionic site of AChE, located near the gorge opening at the protein surface, attracts ACh into the gorge, which uses its structural components noted above to guide the substrate to the active site. The peripheral anionic site may also serve to bind certain cationic ligands and to regulate the activity of AChE in response to changes in ionic strength and excess ACh concentrations. Although some inhibitors of AChE act at the peripheral anionic site, the action of current anti-AChE insecticides is directed against the active site Ser of the catalytic triad (Massoulié *et al.*, 1993; Taylor and Radić, 1994).

AChE exists in two general classes of molecular forms: heteromeric associations of catalytic subunits with structural subunits, and simple homomeric oligomers of catalytic subunits. One heterologous form is a tetramer of catalytic subunits disulphide-linked to a lipid-linked subunit and found on the outer surface of the cell membrane. Some homomeric forms are found as soluble intracellular species or associated with the outer membrane of the cell via attached glycophospholipid (Bourne *et al.*, 1999; Massoulié *et al.*, 1993; Taylor and Radić, 1994).

Biochemistry: mechanism of ACh hydrolysis

As noted above, the primary biological role of AChE is the rapid termination of the neuronal impulse that occurs when ACh is released. Counterbalancing the activation of ACh receptors in post-synaptic cells, AChE hydrolyses ACh via hydrolysis into acetate and choline (Figure 3.4) (Quinn, 1987).

In a single catalytic cycle, the serine hydroxyl group located in the active site of AChE reacts with the carbonyl group of ACh. A transient intermediate (shown in brackets in Figure 3.4) is formed that rapidly loses choline *en route* to forming acetylated AChE. Acetylated AChE represents the essential intermediate of the catalytic sequence; it is unstable and reacts readily with water (topically delivered to the acetylated carbonyl by solvated AChE) to produce the second product, acetate (acetic acid). AChE is regenerated in the process, thus completing the cycle.

Figure 3.4 AChE-catalysed hydrolysis of ACh

Approximately 10 000 molecules of ACh per second are catalytically converted to acetate and choline in the normal operation of this extraordinary enzyme (Quinn, 1987; Taylor and Radić, 1994).

In order to avoid excessive stimulation of the post-synaptic cell, ACh must be destroyed almost as soon as it is released into the synaptic cleft. Neurotransmitter destruction is accomplished by strategic localization of AChE in pre-synaptic and post-synaptic membranes and in the basal lamina in the neuromuscular junction. If AChE is unavailable or inactivated by an anti-AChE agent, then the ACh released from pre-synaptic vesicles will remain at a high concentration in the synaptic cleft leading to excessive and prolonged activation of ACh receptors. This hyperactivity of receptors precipitates overstimulation of post-synaptic cells followed by depression in autonomic ganglia, somatic muscles, and some CNS neurons (Ecobichon, 2001; Taylor, 1996). These molecular and cellular events lead to the untoward effects described in the section below on the toxicological consequences of AChE inhibition.

Major classes of anti-AChE insecticides

The two major classes of insecticides that act as anti-AChE agents are carbamates and organophosphorus (OP) compounds. Although both classes inactivate AChE by covalent attachment at the active site serine, there are important differences in their structure and mechanism of action, discussed in detail below (Ecobichon, 2001; Murphy, 1986; Taylor, 1996). These compounds have undergone extensive development since their inception, and most of the products in use today were introduced decades ago. Therefore, it is doubtful that new variations on the carbamate or OP theme will be employed in the design of future insecticides.

Recently, an ingenious strategy called 'click chemistry' has been devised (Kolb, Finn, and Sharpless, 2001) and extended for creating powerful inhibitors of enzymes (Lewis *et al.*, 2002). The final product is assembled from simple building

blocks using the active site of the enzyme as a template. This approach has produced the most potent reversible inhibitor of AChE ever developed, thus launching a potential new generation of anti-AChE agents that could find uses as both drugs and insecticides. Until such products emerge, however, the established carbamate and OP compounds are the anti-AChE agents that must be considered.

Carbamates: structure and reactivity

Carbamates are an important class of anti-AChE insecticides by virtue of their broad spectrum of activity, ease of preparation, and rapid environmental degradation. The carbamate functional group, also known as a urethane, is shown in Figure 3.5. These compounds may be viewed as mixed ester-amides. A great degree of variation in the groups attached to the carbamate backbone has been explored, particularly in the ester moiety. For example, ester R-groups may be derived from alcohols, phenols, naphthols, or oximes. Some of the simplest and most widely used carbamate insecticides are exemplified by carbaryl, carbofuran, methomyl, and aldicarb (Figure 3.5). The R_1- and R_2-groups attached to the carbamate nitrogen are usually hydrogen and/or methyl; any changes beyond these two simple substituents dramatically alters the reactivity toward AChE and hence the toxicity to target and non-target species (Aldridge and Reiner, 1972; Fukuto, 1990; Kuhr and Dorough, 1976).

Carbamates may react with water via two pathways. Under conditions of basic pH or the presence of nucleophiles, carbamates react at the esteratic bond.

Carbamate

Carbaryl

Carbofuran

Methomyl: X = Me, Y = SMe
Aldicarb: X = H, Y = $C(Me)_2SMe$

Figure 3.5 General structure and selected examples of carbamate insecticides

Carbamate

OH^-, H_2O: ROH + $^-O-C(=O)-N(R_1)R_2$ ⟶ $R_1R_2NH + CO_2$

H^+, H_2O: R_1R_2NH + $R-O-C(=O)-O^-$ ⟶ $ROH + CO_2$

Figure 3.6 Hydrolytic pathways for carbamates

Under acidic conditions, the amide bond is more likely to be cleaved first. Because *N*-carboxylic acids and carbonate monoesters are unstable, the entire carbamate linkage is broken by hydrolysis under alkaline or acidic conditions to yield an alcohol, an amine, and carbon dioxide (Figure 3.6) (Casida, Augustinsson, and Jonsson, 1960; Dittert and Higuchi, 1963).

In the design of many carbamate insecticides, aryloxy and oximate groups at the ester position are employed, because these groups are readily cleaved following hydrolytic reaction with a nucleophile. The stability of the conjugate base of these leaving groups (e.g. phenoxy, naphthyloxy, and oximate anion) drives the reaction (Fukuto, Fahmy, and Metcalf, 1967).

OP compounds: structure, reactivity, and stereochemistry

The term 'organophosphate' is often used as a global synonym for the entire class of OP compounds, including insecticides and nerve agents. Strictly speaking, however, organophosphates comprise only the group of esters and organic acid halides of phosphoric acid. The entire class of OP compounds is much more extensive and includes organophosphonates, organophosphinates, organophosphoramidates, organophosphorothionates, and others (Eto, 1974).

OP insecticides, e.g. acephate, chlorpyrifos, diazinon, malathion, and parathion, are the most widely used agrochemicals for the control of insect pests (Ecobichon and Joy, 1994; Fest and Schmidt, 1973; Gallo and Lawryk, 1991). Accordingly, a substantial amount of information has been compiled about the chemistry and toxicology of these compounds (e.g., Chambers and Levi, 1992; Eto, 1974). Typical OP insecticides vary in the groups attached to phosphorus through sigma bonds. These may include —OR, —SR, and/or —NHR in a variety of combinations to form esters, thiolesters, and/or amides, respectively. The R-groups may be substituted or unsubstituted aryl or alkyl groups. The general structure of OP insecticides, along with specific examples derived from various substitutions of the ligands attached to phosphorus, is shown in Figure 3.7.

Z
‖
R_1–P–X
|
R_2

Parathion: $R_1 = R_2$ = OEt, Z = S, X = *p*-nitrophenoxy
Malathion: $R_1 = R_2$ = OMe, Z = S, X = $SCH(CH_2CO_2Et)CO_2Et$
Acephate: R_1 = OMe, R_2 = SMe, Z = O, X = NHC(O)Me

Figure 3.7 General structure and selected examples of OP insecticides

MeO–P(=S)(OMe)–S–C*H(CO_2Et)(CH_2CO_2Et)

Malathion

MeS–P*(=O)(OMe)–NHC(=O)CH_3

Acephate

Figure 3.8 Structures of malathion and acephate. Chiral centres at carbon and phosphorus denoted by an asterisk

Although most OP insecticides are not chiral, malathion and acephate contain an asymmetric centre at a ligand carbon and the phosphorus atom, respectively (Figure 3.8). Thus, these particular compounds exist as mixtures of enantiomers, and the separate stereoisomers have different reactivities with target and/or biotransformation enzymes and exhibit differential toxicity (Eto, 1974).

One of the three groups or ligands attached to phosphorus via a sigma bond is generally a good leaving group, e.g. the *p*-nitrophenyoxy group in paraoxon (the active metabolite of parathion) or the diethyl thiosuccinyl group in malaoxon (the active metabolite of malathion). The leaving group is displaced by nucleophilic attack of the active site serine of AChE, as described in more detail below. Displacement is favoured if the resulting anion is relatively stable, i.e. if its protonated form is relatively acidic (Eto, 1974). Although this reaction with the active form of an OP insecticide with AChE is often called phosphorylation or organophosphorylation, the term *phosphylation* has been introduced in order to encompass reactions of the enzyme not only with true organophosphates, but with various other subfamilies of OP compounds as well (Shafferman *et al.*, 1996).

Most OP insecticides contain a P═S, or thionate bond. These ubiquitous compounds are included in a major subgroup of OP insecticides referred to by their chemical classification as *phosphorothionates* (DeMatteis, 1989). Phosphorothionates themselves are essentially inactive as anti-AChE agents. They are useful insecticides, however, because the relatively unreactive thionate (P═S) group can be converted to the more reactive oxon (P═O) form (Figure 3.9). This oxidative desulphuration can be carried out by chemical, biological, or environmental

Figure 3.9 Conversion of phosphorothionates to oxons or phosphorothiolates

agents. In biological systems, the reaction is often catalysed by cytochromes P450. The process results in the formation of a P=O bond that is highly polarized relative to the thionate. The presence of the electron-withdrawing, pi-bonded oxygen atom causes a dipole to form rendering the phosphorus atom electrophilic and susceptible to attack by nucleophiles. As such, insecticide oxons are approximately 1000-fold more reactive as anti-AChE agents than their parent phosphorothionates and are far more hydrolytically unstable, especially under conditions of basic pH (Chambers and Levi, 1992; Eto, 1974).

A second and lesser-known conversion of phosphorothionates to oxons occurs when the parent compounds isomerize to form phosphorothiolates (Figure 3.9) (Thompson, 1992). This reaction simultaneously results in the formation of an oxon and a thiolester in the same molecule. Because the R—S ligand in a phosphorothiolate can serve as a leaving group, isomerization not only converts the phosphonothionate to an active AChE inhibitor, but it provides an additional choice for nucleophilic displacement by the serine hydroxyl of the enzyme. Another interesting outcome of this isomerization reaction is that the resultant phosphorothiolates are asymmetric at phosphorus, although the parent phosphorothionate did not originally have a chiral phosphorus atom.

These thiono–thiolo rearrangements can be induced chemically or thermally (Metcalf and March, 1953; Thompson *et al.*, 1989), and to a lesser extent, photochemically (Chukwudebe *et al.*, 1989). Such isomerizations usually are associated with elevated temperatures (80–180°C) or reaction with alkyl halides. However, isomerization may also occur at room temperature over long periods of time when phosphorothionates are stored in certain solvents. The reaction is fastest when the alkyl group being transferred is a methyl (Eto, 1974).

The consequences of the phosphorothionate–phosphorothiolate isomerization are several-fold (Thompson, 1992). First, as noted above, the rearrangement forms an oxon that is approximately 1000-fold more potent as an anti-AChE agent than the parent phosphorothionate. Second, the thiolate group (—SR) formed in the isomerization is a new or additional leaving group that may be displaced during

the phosphylation of serine esterases. Third, the oxons formed can inhibit not only target AChE, but also detoxifying carboxylesterases, thereby potentiating the anti-AChE effect. Fourth, the isomerization usually generates a new centre of asymmetry at the phosphorus atom to yield a mixture of stereoisomers. Overall, isomerization results in an array of reactive impurities that have the potential to exacerbate the toxicity of the parent phosphorothionate. Indeed, the isomerization of malathion to isomalathion has been linked to an episode of mass poisoning of pesticide applicators in Pakistan (Aldridge *et al.*, 1979; Baker *et al.*, 1978).

Because malathion already contains a chiral centre at a ligand carbon, formation of an additional chiral centre at phosphorus via isomerization produces four stereoisomers of isomalathion (Figure 3.10).

The four stereoisomers of isomalathion have been independently synthesized and their mechanisms of inhibition of AChE and other α/β hydrolases analysed. In a series of studies (Berkman *et al.*, 1993a; Berkman, Quinn, and Thompson, 1993b; Doorn *et al.*, 2000, 2001a, 2001b; Jianmongkol *et al.*, 1996, 1999; Thompson, Ryu, and Berkman, 1992), significant differences were found in the anti-AChE activity of the four isomalathion stereoisomers. Depending on the species of AChE, isomalathion stereoisomers with the R_P configuration were 10- to 29-fold better anti-AChE compounds than isomalathions with the S_P configuration. Moreover, AChE inhibited by R_P-isomalathions reactivated with or without oxime, but AChE inhibited by S_P-isomalathion stereoisomers remained refractory to reactivation. Mass spectrometry and molecular modelling studies showed that AChE and other α/β hydrolases inhibited by S_P stereoisomers had undergone ageing or an ageing-type mechanism (see below), and that the primary leaving group differed between R_P and S_P stereoisomers (Doorn *et al.*, 2000, 2001a, 2001b, 2003). Identification of specific OP adducts on AChE and other esterases using mass spectrometry thus proved useful for elucidating a reaction mechanism; however, it also indicates a feasible approach for developing biomarkers of exposure to OP compounds.

Malathion — isomerization → R_PR_C-isomalathion, R_PS_C-isomalathion, S_PR_C-isomalathion, S_PS_C-isomalathion

Figure 3.10 Formation of the four stereoisomers of isomalathion from malathion

Mechanism of inhibition of AChE by carbamates and OP compounds

The mechanism of AChE inhibition by carbamates (Figure 3.11) closely resembles the catalytic hydrolysis of ACh (see Figure 3.4 above) (Wilson, Hatch, and Ginsburg, 1960). Similar to the reaction with substrate, the inhibitor first forms a Michaelis-type complex with the enzyme. The activated serine hydroxyl group in the catalytic triad of the active site then reacts with the carbonyl group of the carbamate to form a carbamoylated AChE. The overall inactivation of the enzyme is characterized kinetically by the bimolecular rate constant of inhibition (k_i), which provides a measure of the inhibitory potency of the compound. When first-order kinetics are observed, the I_{50} may be calculated from the k_i by the relationship, $I_{50} = 0.693/(k_i \times t)$, where t is the time of pre-incubation of inhibitor and enzyme before the addition of substrate, and I_{50} is the concentration of inhibitor resulting in loss of 50 per cent of the enzymatic activity after pre-incubating the enzyme for a fixed time. Kinetic determinations of inhibitory potency are preferable to fixed-time measurements, because they yield a more complete understanding of the inhibitory process (Aldridge and Reiner, 1972; Richardson, 1992; Taylor and Radić, 1994).

In contrast to the acetylated enzyme, methylcarbamoyl AChE ($R_1 = H$, $R_2 = Me$) or dimethylcarbamoyl AChE ($R_1 = R_2 = Me$) is somewhat stable, with a $t_{1/2}$ value for hydrolysis of the dimethylcarbamoyl enzyme of 15–30 min, rendering the carbamoylated AChE unable to hydrolyze ACh for 3–4 h *in vivo* (Taylor, 1996). Enzymatic activity is restored following displacement of the carbamoylated group with water. The kinetics of the hydrolytic reactivation of AChE is characterized by a rate constant, k_3, related to the $t_{1/2}$ for hydrolysis by the relationship, $t_{1/2} = 0.693/k_3$ (Aldridge and Reiner, 1972). The *N*-carboxyamine formed by hydrolysis of the carbamoylated enzyme rapidly degrades to amine and carbon dioxide (Kuhr and Dorough, 1976). Although the k_3 for carbamoylated AChE is substantially larger than that for acetylated enzyme, it usually is significantly smaller than that for phosphylated AChE and becomes an important consideration when assaying blood or tissue samples for AChE inhibition by carbamates (Nostrandt, Duncan, and Padilla, 1993).

Carbamate — Carbamoylated AChE — Reactivated AChE ($+ HNR_1R_2 + CO_2$)

Figure 3.11 Inhibition of AChE by carbamates. k_i = bimolecular rate constant of inhibition; k_3 = rate constant of spontaneous reactivation

Because of the relatively rapid reactivation of carbamoylated AChE, carbamates have sometimes erroneously been considered reversible inhibitors (Aldridge and Reiner, 1972; Main, 1980). A truly reversible inhibitor, e.g. decamethonium, binds to the enzyme through van der Waals and Coulombic forces and dissociates from the enzyme unchanged. Carbamates, are chemically altered when they inhibit AChE, because they undergo nucleophilic displacement of a leaving group, thus becoming covalently attached to the active site serine of the enzyme. Furthermore, as noted above, reactivation of carbamolyated AChE via hydrolysis is not a dissociation of the intact inhibitor, but a chemical reaction that destroys the carbamate group.

OP compounds inhibit AChE by a process analogous to that of the carbamates. Inhibition results from a chemical reaction between the phosphoryl (P=O) moiety of the OP compound and the active site serine hydroxyl group of AChE, form a phosphylated enzyme. As noted above (Figure 3.9), phosphorothionates (P=S) must first be converted to the more reactive oxon form (P=O) via oxidative desulphuration or less commonly by isomerization (Gallo and Lawryk, 1991; Thompson, 1992).

Unlike the acetylated and carbamoylated intermediates formed by reaction of AChE with ACh and carbamates, respectively, the phosphylated enzyme is relatively stable and resistant to hydrolysis (relatively low k_3 and concomitantly high $t_{1/2}$). Consequently, the complete restoration of AChE activity following inactivation by OP compounds is slow (hours to days) (Main, 1980).

When OP compounds inactivate AChE, one of the ligands attached to phosphorus, the primary leaving group (X), is displaced to result in covalent modification of the active site serine (Figure 3.12). The first step in the inactivation is an essentially reversible formation of an enzyme-inhibitor complex (Aldridge and Reiner, 1972; Fukuto, 1990). The second step of inhibition results in the covalent phosphylation of

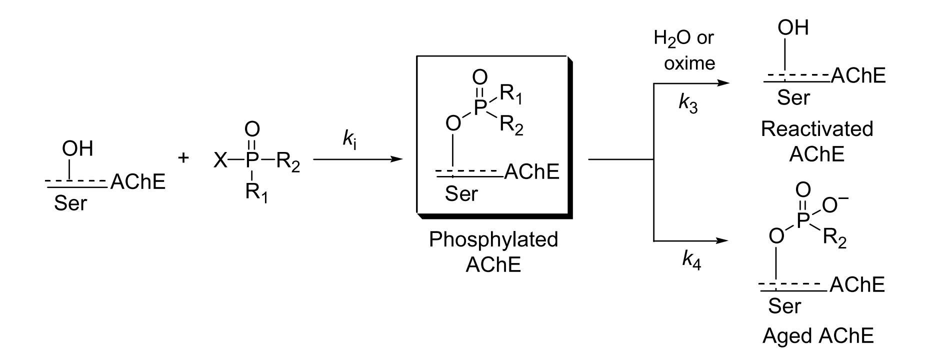


Figure 3.12 Inhibition of AChE by OP oxons. k_i = bimolecular rate constant of inhibition; k_3 = rate constant of spontaneous or oxime-mediated reactivation; k_4 = rate constant of ageing

AChE, typically leading to *O*,*O*-dialkylphosphoryl groups attached to the active site serine hydroxyl (Chiu, Main, and Dauterman, 1969). As with the carbamates, the overall kinetic process that accounts for the covalent modification of AChE by OP compounds is characterized by k_i, a bimolecular rate constant that provides a measure of the ability of a compound to inactivate the enzyme. Inhibitors with k_i values ($M^{-1}\,min^{-1}$) $< 10^2$ are considered weak (e.g. phosphorothionates before activation to oxons), from 10^3 to 10^4 moderate (e.g. malaoxon and carbaryl), and $>10^5$ strong (e.g. paraoxon and nerve agents). It is interesting to note that some carbamates that are used as drugs rather than as insecticides, e.g. physostigmine and neostigmine, are highly potent inhibitors, with k_i values of about 10^6 and $10^7\,M^{-1}\,min^{-1}$, respectively (Aldridge and Reiner, 1972).

Inhibition of AChE by carbamates is followed by a single post-inhibitory pathway involving a relatively rapid restoration of enzyme activity characterized by a rate constant of reactivation, k_3 (Figure 3.11). In contrast, phosphylated AChE can undergo more than one post-inhibitory pathway; two of these are commonly described as reactivation or ageing, with their associated rate constants k_3 and k_4, respectively (Figure 3.12). The more general situation for the variety of possible OP compound–AChE conjugates, however, is somewhat more complex and it may be preferable to consider the post-inhibitory pathways as reactivation and non-reactivation, with ageing as a specific case of non-reactivation (Berkman, Quinn, and Thompson, 1993b). These post-inhibitory pathways for phosphylated AChE are first summarized and then described in more detail below.

- *Reactivation*. There are two main types of reactivation of phosphylated AChE, spontaneous or oxime-induced. Spontaneous reactivation results when water breaks the phosphylserine bond. Oxime-induced reactivation results when an oxime antidote, e.g. 2-pralidoxime methiodide (2-PAM), is used to enhance the rate of cleavage of the phosphylserine bond.

- *Non-reactivation*. Non-reactivation results when all or part of the activity of a phosphylated AChE cannot be restored, owing to a process other than classical ageing. These processes include steric occlusion of the active site by bulky ligands attached to phosphorus, denaturation or conformational change of the protein, chemical modification of a secondary residue (other than the active site serine) on AChE, or modification of the phosphyl group (e.g. via hydrolysis) to render the phosphylated AChE intractable to reactivation.

- *Ageing*. Ageing is a specific mechanism of non-reactivation involving dealkylation of the phosphyl moiety to yield a phosphyl monoanion that is still covalently attached to the active site serine of AChE.

The mechanism of spontaneous reactivation proceeds via nucleophilic attack of water on the phosphorus atom, cleaving the serine phosphylester bond in a

substitution process that ejects AChE as a leaving group. The ability of AChE to recover spontaneously from inhibition by an OP compound depends upon the type of AChE, pH, ionic strength, temperature, and, most importantly, the nature of the phosphyl moiety (Ashani *et al.*, 1995; Clothier, Johnson, and Reiner, 1981; Fisher, Crane, and Callaghan, 2000; Wallace and Herzberg, 1988; Wilson *et al.*, 1992; Wong *et al.*, 2000). The rate of spontaneous reactivation is greatest when the phosphyl group contains less bulky ligands (R_1, R_2; Figure 3.12). For example, dimethoxy phosphorylated AChE ($R_1 = R_2 = OMe$) reactivates with rates that are 15- and 30-fold faster than the corresponding diethoxy ($R_1 = R_2 = OEt$) and di-*n*-propoxy ($R_1 = R_2 = n$Pr) phosphylated AChE, respectively (Eto, 1974; Gallo and Lawryk, 1991).

In the 1950s, Wilson and co-workers explored the use of strong nucleophiles such as oximes to break the phosphylserine bond of AChE inhibited by OP compounds (Wilson and Ginsberg, 1955; Wilson, Ginsburg, and Quan, 1958). This pioneering work led to the discovery that oximes attached to positively charged 'carrier' groups (to mimic the quaternary amine of ACh) greatly accelerate the reactivation of phosphylated AChE. Thus, these compounds have become universal antidotes for AChE inactivated by OP agents (Froede and Wilson, 1971; Wilson *et al.*, 1992). Examples of oximes currently used include pyridine-2-aldoxime methiodide (2-PAM) and 1,1′-trimethylenebis(pyridinium-4-aldoxime) dibromide (TMB-4) (Figure 3.13).

Oxime reactivation of AChE inhibited by an OP compound is proposed to proceed via the mechanism depicted in Figure 3.14 (Aldridge and Reiner, 1972; Luo *et al.*, 1999). The process is similar to that of spontaneous reactivation and dependent upon the same factors, e.g. enzyme source, pH, and the nature of the ligands attached to the phosphorus atom. Oxime-induced reactivation is more effective when the phosphorus ligands are small and less effective when at least one of the ligands is bulky. For example, the oxime-induced reactivation rate constant, k_3, is 60-fold greater for dimethyl phosphorylated AChE ($R_1 = R_2 = OMe$) than the diethyl ($R_1 = R_2 = OEt$) (Clothier *et al.*, 1981) and diisopropyl phosphoryl conjugates (Taylor, 1996). The size of the ligands in relation to the dimensional

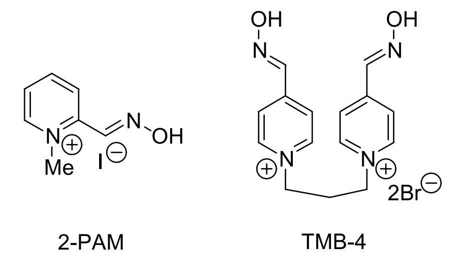


Figure 3.13 Structures of the oxime reactivator agents 2-PAM and TMB-4

Figure 3.14 Mechanism of oxime-induced reactivation of OP-inhibited AChE. k_3 = rate constant of oxime-mediated reactivation

constraints of the gorge may limit access of the nucleophile to the active centre as well as egress of the displaced dialkyl phosphoryl moiety (Ashani *et al.*, 1995; Wong *et al.*, 2000).

In parallel with displacement of the phosphorus ester group from the active site serine hydroxyl, a phosphylated-oxime product results (Figure 3.14). Some phosphylated oximes are more potent inhibitors than the original OP compound, and rephosphylation can occur resulting in a steady state between reactivation and reinhibition. Fortunately, the phosphylated products of therapeutically useful oximes are labile to hydrolysis, leading to innocuous phosphyl monoanions (Eto, 1974).

Although oxime reactivator agents can cleave the phosphyl–serine bond, effective restoration of AChE activity is the result of several factors. In addition to the inhibitory potency and stability of the phosphyl oxime noted above, other important parameters include the approach trajectory of the nucleophile with respect to the phosphyl–AChE conjugate, stereochemistry of the phosphorus group, size of the ligands attached to phosphorus, and the degree to which non-reactivation processes, including ageing, occur. In the case of ageing, the resultant phosphyl anion attached to the active site serine resists displacement and the aged enzyme is refractory to reactivation. The resistance of aged AChE to reactivation may be due in part to charge repulsion of attacking nucleophiles and also to adduct-stabilizing conformational shifts induced in the protein by the ageing process (Amitai *et al.*, 1982; Ashani, Gentry, and Doctor, 1990; Millard *et al.*, 1999b).

Non-reactivation is a post-inhibitory phenomenon that results in the inability to restore all or part of the activity of phosphylated AChE either spontaneously or by treatment with oximes or other nucleophiles. Such resistance to reactivation may have one or more causes. Possible mechanisms include steric occlusion of the active site (Hosea, Berman, and Taylor, 1995; Wong *et al.*, 2000), modification of tertiary protein structure (Grubič *et al.*, 1995; Morel *et al.*, 1999; Šentjurc *et al.*, 1999), covalent modification of a residue other than serine (Mullner and Sund, 1980), and displacement of a phosphyl ligand via hydrolysis (Thompson, Ryu, and Berkman, 1992). These post-inhibitory processes result in a time-dependent loss of the ability of the inhibited enzyme to be reactivated, but they do not involve a secondary

dealkylation of the phosphyl–AChE adduct. Thus, these modes of non-reactivation are distinct from the specific case of ageing, described in more detail below.

Ageing occurs when the phosphyl group is dealkylated, resulting in a phosphylated oxyanion conjugate that is resistant to both spontaneous and oxime-induced reactivation. As shown in Figure 3.12, an OP compound can inhibit AChE to yield a phosphyl–AChE conjugate that then loses one of its ligands via dealkylation. Although the highly polarized P=O bond makes OP compounds susceptible to nucleophilic attack by both the serine hydroxyl during inhibition and subsequently to reactivating nucleophiles, e.g. H_2O or oximes (Hosea *et al.*, 1995; Wong *et al.*, 2000), ageing typically represents a reaction at a ligand carbon (Shafferman *et al.*, 1996).

There are many factors that contribute to the propensity of a given OP–AChE conjugate to undergo ageing, including the type of ligand (ester, thiolester, or amide), branching at the alkyl group, and competing reactivation reaction kinetics (Clothier, Johnson, and Reiner, 1981). For example, dimethoxy phosphyl conjugates of bovine erythrocyte AChE (Figure 3.12, $R_1 = R_2 =$ OMe) spontaneously reactivate and age more rapidly than diethoxy phosphyl conjugates (Figure 3.12, $R_1 = R_2 =$ OEt). In general, phosphyl conjugates containing thiolester bonds (e.g. $R_1 =$ SMe; $R_2 =$ OMe) spontaneously reactivate and age more rapidly than analogues containing only oxyester bonds. The ageing half-lives of most agrochemical OP compounds that usually contain simple unbranched ligands range from 2 to 60 h, whereas the OP chemical warfare agent soman, with its branched pinacolyl ligand, ages in less than 10 min (Eto, 1974).

An additional explanation for the ageing phenomenon is that, compared with the neutral phosphyl conjugate of AChE, the phosphyl anion that results from dealkylation is preferentially stabilized within the active site. The X-ray crystal structures of phosphylated AChE from *Torpedo californica* combined with kinetic data for a number of AChEs modified by site-specific mutagenesis reveal a number of structural features that appear to facilitate the ageing process (Millard *et al.*, 1999b). In particular, as discussed above, the region defined by the amide backbone hydrogens of residues Gly118, Gly119, and Ala201 (Sussman *et al.*, 1991) forms a stabilizing cavity within the active site, termed the oxyanion hole. This structure is believed to stabilize the phosphyl oxygen of an OP inhibitor by donating hydrogen bonds (Ordentlich *et al.*, 1998). The oxyanion hole is also thought to facilitate the reactions of inhibition and reactivation, as well as the mechanisms associated with ageing (Hosea *et al.*, 1995; Wong *et al.*, 2000).

The resolved crystal structures from neutral and aged OP conjugates of AChE have the phosphyl oxygen positioned within hydrogen bonding distance of the oxyanion hole. It is possible, therefore, that the oxyanion hole can stabilize the bound OP adduct both before and after the ageing reaction has occurred (Millard *et al.*, 1999a, 1999b; Ordentlich *et al.*, 1999). Ageing may enhance the interaction between the OP conjugate and the oxyanion hole by placing the formal negative charge generated during the dealkylation reaction partially, or entirely, in the dipolar oxyanion hole (Millard *et al.*, 1999b).

As discussed above, the negative charge introduced by the loss of an alkyl group during the ageing process (Figure 3.12) has been thought to impose a significant barrier to subsequent nucleophilic dephosphylation due to charge repulsion (Westheimer, 1987). Electrostatic repulsion, however, is an insufficient explanation for the profound resistance of aged AChE to reactivation, given that the presence of a negative charge on simple phosphorus diesters retards the rate of nucleophilic attack by less than 100-fold (Kirby and Lancaster, 1970). The fact that protein denaturation of aged AChE permits significant base-catalysed dephosphylation (Segall *et al.*, 1993) suggests that the resistance of aged AChE to reactivation is due, in part, to specific interactions between the dealkylated phosphyl moiety and the aforementioned stabilizing effects of AChE. A practical result of ageing is that once it occurs, it prevents reactivation of AChE by oximes, thus limiting the therapeutic regimens that may be used in cases of poisoning by OP compounds (Wilson *et al.*, 1992).

Toxicological consequences of AChE inhibition

Inactivation of a sufficient amount of AChE resulting from exposure to carbamate or OP insecticides results in the rapid accumulation of ACh in neuromuscular junctions and neural synapses. The excess ACh results in overstimulation of nicotinic and muscarinic receptors of autonomic organs and skeletal muscles in the PNS, as well as cholinergic receptors (predominantly muscarinic) of the CNS. Numerous signs and symptoms (Table 3.1) elicited by exposure to anti-AChE insecticides can be explained by considering the locations of nicotinic and muscarinic receptors throughout the CNS and PNS and the consequences of their activation (Ecobichon, 2001; Taylor, 1996).

Table 3.1 Signs and symptoms resulting from AChE inhibition

Nervous system	Receptor type	Target tissue	Signs and symptoms
Central	Muscarinic and nicotinic	Brain	Confusion, depression, slurred speech, hypothermia, lethargy, tremors, loss of reflexes, paralysis, convulsions, coma
Peripheral	Muscarinic	Heart, blood vessels, lungs, bladder, GI tract, exocrine glands	Bradycardia, hypotension, bronchial constriction, excess secretions, incontinence, nausea, vomiting, diarrhoea, salivation, miosis, sweating, blurred vision, lacrimation
	Nicotinic	Heart and blood vessels, skeletal muscle	Tachycardia, hypertension, fasciculations, ataxia, weakness, involuntary twitching, convulsions, paralysis

When the concentration and residence time of ACh is increased in the synapse due to AChE inhibition, a persistent depolarization of the motor endplate is observed. The decay of endplate currents or potentials resulting from spontaneous release of ACh is prolonged from 1–2 ms to 5–30 ms. This extended duration of currents indicates that the transmitter activates multiple receptors before diffusing from the synapse. Excessive depolarization of the endplate, resulting from slowly decaying endplate potentials, leads to a diminished capacity to initiate coordinated action potentials. In a fashion similar to depolarizing blocking agents, fasciculations and muscle twitching are observed initially with AChE inhibition, followed by flaccid paralysis (Taylor, 1996).

The consequences of AChE inhibition also differ among synapses. At post-ganglionic parasympathetic effector sites, AChE inhibition enhances or potentiates the action of ACh released by nerve stimulation. In part, this facilitation is a consequence of receptor stimulation extending over a large area from the point of transmitter release, owing to inadequate removal of surplus ACh by AChE. Similarly, ganglionic transmission is enhanced by anti-AChE agents. Given that atropine and other muscarinic antagonists are moderately effective antidotes against the central cholinergic toxicity of anti-AChE insecticides (see below), at least some CNS manifestations result from excessive muscarinic stimulation (Ecobichon, 2001; Taylor, 1996).

Massive acute exposures to anti-AChE agents or prolonged exposures to doses sufficient to cause chronic endplate ACh excess can lead to muscle weakness through downregulation of nicotinic receptors or muscle necrosis (Ecobichon and Joy, 1994). The so-called intermediate syndrome (Senanayake and Karalliedde, 1987) appears to be related to profound and protracted AChE inhibition rather than arising from a unique mechanism, and it is not a form of delayed neuropathy (OPIDN) (De Bleecker, 1995). A variety of other neurological or behavioural sequelae has been reported to result from exposure to anti-AChE insecticides (Ecobichon, 2001; Marrs, 1993), but the mechanisms of such effects await elucidation.

Therapy for cholinergic toxicity

Cholinergic toxicity from anti-AChE insecticides is one of the few types of poisoning that may be treated by specific antidotes. These include ACh receptor blockers (e.g. atropine) to reduce overstimulation of receptors following inhibition of AChE by either carbamate or OP insecticides, and oximes (e.g. 2-PAM) to reactivate AChE inhibited by OP compounds. Prophylaxis against anticipated exposure to nerve agents is also a specific intervention that involves purposeful short-term inhibition of AChE with a carbamate (e.g. pyridostigmine) to prevent phosphylation and long-term suppression of AChE activity (Ellenhorn *et al.*, 1997).

Non-specific supportive measures may be applied to augment or replace pharmacological antagonists to counteract certain effects of anti-AChE intoxication.

The clinical manifestations of severe cholinergic toxicity are dominated by respiratory insufficiency and artificial ventilation is a major therapy (Sungar and Guven, 2001). In the acute phase of a cholinergic crisis, convulsions may be controlled with anticonvulsants, e.g. diazepam (Taylor, 1996).

In the following sections, two types of treatments for anti-AChE insecticide poisoning are presented. In order to institute proper therapy and monitor its effectiveness, it is important to have obtained as much information as possible to confirm the diagnosis of cholinergic toxicity and its ætiology. This would include ascertaining, for example, through taking a medical history, if the cholinergic signs and symptoms are due to carbamate or OP intoxication, because the use of oximes is generally contraindicated for carbamate poisoning (Ecobichon, 2001). Determinations of plasma BChE and erythrocyte AChE activities should be done as important concomitants of the history and clinical diagnosis, and to monitor the progress of recovery, whether by biological replacement of enzyme or reactivation by oxime treatment. Unfortunately, there may be a poor correlation between clinical expression of cholinergic toxicity and plasma BChE or erythrocyte AChE activities (especially in mild cases), but frank toxicity will usually be associated with a marked depression of one or both of these values soon after the poisoning episode (Bobba *et al.*, 1996; Lotti, 1995). Cardiac monitoring is advised for anti-AChE exposures resulting in substantial reduction in blood cholinesterase activities (Saadeh, Farsakh, and al-Ali, 1997).

Muscarinic ACh receptor antagonism

Atropine is the principal muscarinic ACh receptor antagonist used to counteract the cholinergic toxicity of anti-AChE insecticides (Ellenhorn *et al.*, 1997). Because the action of atropine is independent from the mechanism of AChE inactivation, it may be used to treat poisoning by both OP and carbamate insecticides. By virtue of its structural similarity to ACh and binding to M_1 and M_2 muscarinic receptors without activating them, atropine (Figure 3.15) can inhibit the interaction of the neurotransmitter with this subset of cholinergic receptors via competitive antagonism (Weiner, 1985). This anticholinergic agent thus counteracts the muscarinic effects noted in Table 3.1. Given that atropine is primarily a muscarinic blocker, the nicotinic effects listed in Table 3.1 will not be reversed.

Me N O O CH_2OH

Figure 3.15 Structure of atropine

The dose and timing of administration of atropine will depend on the distribution, severity, and duration of AChE inhibition, and the concomitant populations of muscarinic receptors that are overstimulated. Patients exhibiting preliminary signs of cholinergic crisis are given 1–5 mg of atropine (adult) by either intramuscular or intravenous injection. Severely poisoned patients may receive 20–30 mg of atropine over several hours. Successful atropinization may be monitored by drying of secretions and reversal of miosis (Ecobichon, 2001; Taylor, 1996).

Oxime reactivation of inhibited AChE

Oximes, e.g. 2-PAM and TBM-4 (Figure 3.13), are used to reverse the covalent modification of AChE by OP compounds (Figure 3.14). The principal difficulty in using oxime antidotes is that they must reach the phosphylated AChE conjugate while it is still possible to reactivate the enzyme: oxime therapy must precede ageing or other non-reactivation reactions. Because of this limitation and the equimolar requirement for displacement of the OP moiety from AChE, relatively large doses (1 g, intravenously, for adults) of 2-PAM must be given as early as possible after establishing a diagnosis of OP insecticide intoxication (Ecobichon, 2001; Wilson, 1992).

Oximes have some anti-AChE activity of their own and carbamoylated AChE undergoes spontaneous reactivation quickly compared with phosphylated AChE. Thus, although oximes can reactivate carbamoylated AChE, and in the case of selected compounds have been shown to be of therapeutic benefit, the use of oximes as antidotes against carbamate poisoning is generally not recommended (Burgess, Bernstein, and Hurlbut, 1994; Kurtz, 1990; Wilson *et al.*, 1992).

Because most of the therapeutically employed oximes contain positively charged quaternary amines, they would be expected not to cross the blood–brain barrier and, therefore, be of most use for reactivation of AChE in the PNS. Moreover, although the active form of the nucleophilic moiety in these agents is thought to be the oxime anion, zwitterionic species incorporating the quaternary amine cation and oxime anion should also have difficulty diffusing into the CNS. Nevertheless, reactivation of brain AChE and improvement of CNS cholinergic signs following oxime treatment has been reported (Lotti and Becker, 1982; Wilson *et al.*, 1992). Such contrasts between theoretical predictions and experimental or clinical observations indicate that we still have much to learn about the mechanisms of toxicity and treatment pertaining to anti-AChE insecticides.

Regulatory aspects of anti-AChE insecticides

OP and carbamate insecticides are unusual compounds with respect to regulatory toxicology in that there is a convenient and easily assayed biomarker of their effect,

namely inhibition of the activity of AChE or BChE. Reference doses (RfDs), e.g. acceptable daily intakes (ADIs) or acute reference doses (ARfDs), are typically determined by dividing the 'no observed adverse effect levels' (NOAELs) in animal studies (or less commonly in human studies) by one or more uncertainty factors (UFs). By default, a UF (also called a safety factor) of 10-fold is used for each source of unpredictability, e.g. animal-to-human extrapolation or variability within the human population. When there is sufficient information from, for example, mechanistic or pharmacokinetic studies, the default UF may be multiplied by a modifying factor or replaced by a factor derived from relevant data (Faustman and Omenn, 2001; Pope, 1999). Unlike the situation with other pesticides, where the critical effect determining the NOAEL is a clear adverse clinical, histopathological or biochemical outcome in the animals, with anti-AChE compounds it is most often inhibition of AChE or BChE (Carlock *et al.*, 1998). Selected RfDs derived in this way for OP pesticides by the Food and Agriculture Organization/World Health Organization Joint Meeting on Pesticide Residues (FAO/WHO JMPR) are listed in Table 3.2. These values may vary by several orders of magnitude, e.g. from mevinphos to malathion.

In the international arena, most regulatory authorities consider BChE inhibition to be simply a biomarker for assessment of exposure and not an injurious effect (Carlock *et al.*, 1998). As discussed earlier in this chapter, there are individuals with mutations in the gene for BChE, who have diminished or no activity of this enzyme. However, these people do not suffer ill health, except in the special circumstance of succinylcholine being used as a muscle relaxant during anaesthesia (Lockridge, 1992). Episodes of adverse reactions to muscle relaxants arising from exposure to anti-AChEs are extremely rare (Selden and Curry, 1987; Sparks, Quistad, and Casida, 1999). Indeed, such cases are virtually confined to surgical procedures on animals that had been treated with OP ectoparasiticides (Himes *et al.*, 1967; Keller and Müller, 1979; Short, Cuneio, and Cupp, 1971). Cocaine is also metabolized by BChE and drug abusers with low levels of the active enzyme may be predisposed to increased harmful effects from the illicit use of this alkaloid (Hoffman *et al.*, 1992a, 1992b). Nevertheless, human case reports of cocaine toxicity potentiated by anti-AChE insecticides are lacking. Thus, the consensus view has generally been not to treat plasma BChE inhibition as adverse.

In animal studies, where both brain and erythrocyte AChE are measured, there has been some discussion as to whether inhibition of the latter by itself should be considered adverse. The function of erythrocyte AChE is obscure and its inhibition *per se* would seem to be without adverse clinical effect (Chen *et al.*, 1999). There is concern, however, that sole reliance on inhibition of brain AChE would be insufficiently predictive of effects at peripheral sites of cholinergic action, especially the neuromuscular junction, given that this structure is not protected by the blood–CNS barrier. Most OP insecticides are quite lipophilic, however, and would be capable of readily gaining access to the CNS as well as peripheral sites (Chambers and Carr, 1993; Vale, 1998). Moreover, nervous tissue can resynthesize AChE, whereas

Table 3.2 JMPR RfDs for selected OP pesticides[a]

Compound	ADI ($mg\,kg^{-1}\,day^{-1}$)	ARfD (mg/kg)	Study/studies determining ADI; UF	Effect(s) determining NOAEL in study determining ADI	Reference
Azinphos-methyl	0.005		Rat multigeneration; 100	Impaired fertility and pup viability	FAO/WHO (1992)
Cadusafos	0.0003		Rat multigeneration; 100	Impaired weight gain; RBC AChE inhibition in F_1 generation	FAO/WHO (1992)
Chlorfenvinphos	0.0005		Rat multigeneration; 100	Post-implantation and breeding losses; brain AChE inhibition	FAO/WHO (1995)
Chlorpyrifos	0.01	0.1	Human; 10 Mouse, rat, dog; 100	RBC AChE inhibition Brain AChE inhibition	FAO/WHO (2000)
Chlorpyrifos-methyl	0.01		Human; 10	No adverse effect	FAO/WHO (1993)
Diazinon	0.002	0.03	Human; 10	No adverse effect	FAO/WHO (1994, 2002)
Dimethoate[b]	0.002		Rat multigeneration; 500	Impaired pregnancy rate	FAO/WHO (1997)
Disulfoton	0.0003	0.003	Dog, 2-year; 100	Brain AChE inhibition	FAO/WHO (1992, 1997)
Ethoprophos	0.0004	0.05	Rat, 2-year and multigeneration; 100	Brain AChE inhibition	FAO/WHO (2000)
Fenamiphos	0.0008	0.0008	Dog, 1-year; 100	Brain AChE inhibition	FAO/WHO (1998)
Fenitrothion	0.005	0.04	Rat, 2-year; 100	Brain and RBC AChE inhibition	FAO/WHO (2001a)

Fenthion	0.007	0.01	Human; 10	No adverse effect; plasma BChE, but not RBC AChE inhibition at highest dose tested	FAO/WHO (1996, 1998)
Malathion	0.3		Rat, 2-year; 100	Decreased survival; body weight gain; brain AChE inhibition; increased liver, kidney and thyroid/parathyroid weights; focal degeneration in olfactory epithelium	FAO/WHO (1998)
Methidathion	0.001	0.01	Dog, 90-day and 1-year; 100	Cholestasis; elevated serum aminotransferase, alkaline phosphatase and sorbitol dehydrogenase	FAO/WHO (1993, 1998)
Mevinphos	0.0008	0.003	Human; 20	RBC AChE inhibition	FAO/WHO (1997)
Monocrotophos	0.0006	0.002	Human; 10	No adverse effect; plasma BChE inhibition	FAO/WHO (1994, 1996)
Parathion	0.004	0.01	Rat, 2-year; 100	Retinal atrophy; brain AChE inhibition	FAO/WHO (1996)
Parathion-methyl	0.003	0.03	Rat, 2-year; 100	Retinal atrophy; sciatic nerve demyelination; reduced body weight gain; brain AChE inhibition	FAO/WHO (1996)
Phorate	0.0005		Dog, 1-year; 100	Clinical signs, brain AChE inhibition	FAO/WHO (1997, 1995)
			Rat, 2-year; 100	Brain AChE inhibition	

continues overleaf

Table 3.2 *(continued)*

Compound	ADI (mg kg^{-1} day^{-1})	ARfD (mg/kg)	Study/studies determining ADI; UF	Effect(s) determining NOAEL in study determining ADI	Reference
Phosalone	0.02	0.3	Rat, 2-year; 100	Brain AChE inhibition	FAO/WHO (1998, 2002)
Phosmet	0.01	0.02	Rat multigeneration; 100	Reduced mating and fertility	FAO/WHO (1995,1999)
Pirimiphos-methyl	0.03		Human; 10	No adverse effect; no effect on plasma BChE or RBC AChE	FAO/WHO (1993)
Pyrazophos[c]	0.004		Dog, 2-year; 100	Reduced body weight gain; RBC AChE inhibition; calcification in the kidney	FAO/WHO (1993)
			Rat multigeneration; 100	Increased thymus weight and lymphocyte count in the third generation	
Triazophos	0.001		Human; 10	No adverse effect; plasma BChE inhibition	FAO/WHO (1992, 1994)
Trichlorfon (metrifonate[d])	0.02		Human; 10	RBC AChE inhibition	FAO/WHO (2001b)

[a]Abbreviations: AChE, acetylcholinesterase; ADI, acceptable daily intake; ARfD, acute reference dose; BChE, butyrylcholinesterase; JMPR, joint meeting on pesticide residues; NOAEL, no observed adverse effect level; OP, organophosphorus; RBC, red blood cell (erythrocyte); RfD, reference dose; UF, uncertainty (safety) factor.
[b]Expressed as the sum of dimethoate and omethoate as dimethoate.
[c]A fungicide.
[d]International non-proprietary name (INN), when used as a human or veterinary drug.

erythrocytes cannot; therefore, it is conceivable that in long-term, repeated-dose animal studies, using erythrocyte AChE as a surrogate for peripheral nervous AChE might overestimate the degree of inhibition (Lotti, 1995). Indeed, both acute and subchronic studies of OP insecticides indicate that erythrocyte AChE inhibition is more sensitive than brain AChE inhibition (Chen *et al*., 1999; Sheets *et al*., 1997). Another issue that has given rise to some discussion is the degree of AChE inhibition that is considered to be biologically significant; traditionally, the threshold for biologically significant AChE depression has been taken to be 20 per cent (for a discussion of these regulatory issues see FAO, 1999).

Interpretation of studies of blood esterase inhibition can be difficult, particularly if it is not clear whether AChE or BChE activity is being measured. It is important to stipulate the enzyme substrate along with other assay conditions, including whether the enzyme source was erythrocytes, plasma, or whole blood. Even when all these specifications are given, comparison of results across species can be problematic, especially for plasma, because AChE and BChE are found in different relative proportions and absolute amounts in this compartment. For example, human plasma contains almost exclusively BChE, but the ratio of BChE to soluble AChE is approximately 1:1 in rat plasma and 7:1 in dog plasma (Carlock *et al*., 1998; Lotti, 1995).

Changes in addition to AChE and/or BChE inhibition are sometimes seen at the doses determining the NOAEL, and these findings may impinge on the regulatory status of particular pesticides. Thus, dichlorvos was considered to be carcinogenic by the International Agency for Research on Cancer (IARC, 1991), although this opinion has been the subject of considerable debate (Mennear, 1994, 1998; Van Maele-Fabry, Laurent, and Willems, 2000). With malathion, extremely high doses are tolerated in mammals, and changes in organ weights and damage to the olfactory epithelium are observed (FAO/WHO, 1998; Table 3.2). With some anti-AChEs, effects are seen in multigeneration studies. These are not necessarily indicative of specific reproductive toxicity and may arise from other anti-AChE effects, including overt maternal toxicity (Astroff, Freshwater, and Eigenberg, 1998).

Most pesticide regulatory authorities require that tests for the potential of OP compounds to cause OPIDN are carried out in hens (OECD, 1995a, 1995b; USEPA, 1998) and that NTE assays (Kayyali *et al*., 1991) are performed as part of this assessment. In order to cause OPIDN, an OP compound must inhibit a critical fraction (>70 per cent) of NTE in the nervous system, and the inhibited NTE must be capable of undergoing ageing to yield a negatively charged phosphylated NTE (Davis, Johnson, and Richardson, 1985). The structures of OP insecticides would enable them to undergo ageing if they were capable of inhibiting NTE in sufficient amounts before causing death by cholinergic toxicity following AChE inhibition. Hence, the relative inhibitory potency (RIP) of direct-acting OP insecticides or their active oxon metabolites against AChE versus NTE *in vitro* has been used successfully to predict the potential of these compounds to bring about delayed

Table 3.3 JMPR RfDs for selected carbamate insecticides[a]

Compound	ADI ($mg\,kg^{-1}\,day^{-1}$)	ARfD (mg/kg)	Study determining ADI; UF	Effect(s) determining NOAEL in study determining ADI	Reference
Aldicarb	0.003	0.003	Human; 10	RBC AChE inhibition	FAO/WHO (1993)
Carbaryl	0.008	0.2	Mouse, 2-year; 2000	Vascular tumours seen at lowest dose tested	FAO/WHO (2002)
Carbofuran	0.002		Dog, 4-week; 100	RBC AChE inhibition	FAO/WHO (1997)
Methiocarb	0.02	0.02	Dog, 2-year; 100	Clinical signs (tremor, weakness in hind legs)	FAO/WHO (1999)
Methomyl	0.02	0.02	Human; 5	RBC AChE inhibition; salivation	FAO/WHO (2002)

[a]Abbreviations: AChE, acetylcholinesterase; ADI, acceptable daily intake; ARfD, acute reference dose; BChE, butyrylcholinesterase; JMPR, joint meeting on pesticide residues; NOAEL, no observed adverse effect level; RBC, red blood cell (erythrocyte); RfD, reference dose; UF, uncertainty (safety) factor.

neuropathy *in vivo* (Kropp and Richardson, 2003; Lotti and Johnson, 1978; Makhaeva *et al.*, 1998; Richardson, 1992; Richardson *et al.*, 1993). It is apparent that when the RIP, calculated either as the [k_i(AChE)/k_i(NTE)] ratio or the [I_{50}(NTE)/I_{50}(AChE)] ratio, is >1, the inhibitor has a greater inhibitory potency against AChE than NTE. When AChE inhibition is favoured over NTE inhibition, the compound would have a greater tendency to produce acute cholinergic toxicity rather than OPIDN. In fact, RIP values >1 correlate with doses of parent compound greater than the LD_{50} being required to produce OPIDN. These tests and insights have ensured that OP pesticides with the potential to cause delayed neuropathy at sublethal doses are not used in agriculture (Mileson *et al.*, 1998; Moretto and Lotti, 1998). Thus, although human cases of poisoning by compounds such as methamidophos have been associated with delayed polyneuropathy, these episodes involved doses that caused severe cholinergic toxicity (Bertolazzi *et al.*, 1991; Sun, Zhou, and Xue, 1998).

In the case of carbamate anti-AChE insecticides, the ADI is often equal to the ARfD. This situation arises because enzyme inhibition and cholinergic toxicity are rapidly reversible and usually no effects are found in subchronic studies that are not found in single dose studies (Table 3.3). An interesting exception is that the JMPR imposed a 2000-fold safety factor in allocating the ADI for carbaryl, because of the occurrence of vascular tumors in animal studies (FAO/WHO, 2002).

An important practical issue in the laboratory evaluation of carbamate insecticides arises from the relatively rapid spontaneous reactivation rate (high k_3 value) of carbamoylated AChE. Because of this property, it is important to ensure that *ex vivo* restoration of enzyme activity does not occur before assay when taking blood samples from animals treated with carbamates (Nostrandt, Duncan, and Padilla, 1993). Thus, as we have seen with the OP insecticides, a thorough knowledge of the nature of the interaction between carbamates and their intended target enzyme is an essential component of the appropriate risk assessment of these compounds.

In conclusion, it should be noted that the regulation of anti-AChE insecticides is an ongoing and evolving process. Such procedures depend critically on good judgment based on careful weighing of accurate and relevant information derived from sound science (Herrman, 1993; Herrman and Younes, 1999; Jun-Shi, 2001). In this way, as our understanding of the chemistry and biology of these products continues to increase, so will our ability to use them wisely for the further global advancement of agriculture and public health.

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4 Toxicology of Pyrethrins and Synthetic Pyrethroids

David E. Ray

Usage and human exposure

The insecticidal properties of dried chrysanthemum flower heads have been recognized for at least two thousand years, and the chemical basis of this activity was first investigated by Staudinger and Ruzicka (1924). The insecticidal properties reside in a mixture of esters of chrysanthemic or pyrethric acids with pyrethrolone, cinerolone, or jasmolone alcohols. These natural pyrethins are unstable when pure, but can be used as practical insecticides in suitable formulations. The natural esters all take the (1*R*) *trans* conformation at the two asymmetric carbons of the cyclopropane ring and the *S* configuration of the alcohol. The first synthetic pyrethroid to reach commercial production was bioresmethrin (Elliott *et al.*, 1967), followed by permethrin, fenvalerate, cypermethrin, and deltamethrin (Figure 4.1). The incorporation of the cyano group on the alcohol introduced the possibility of eight isomeric forms. Some pyrethroids are marketed as racemic mixtures (e.g. permethrin) and others as single isomers (e.g. deltamethrin). Since their introduction in the 1970s, the synthetic pyrethroids have made steady progress as agricultural pesticides, most having the great advantage over the pyrethrins of ultra-violet light stability, which permits outdoor use under tropical conditions. A list of commonly used pyrethroids is given in Table 4.1. The pesticidal activity of both pyrethrins and pyrethroids usually is enhanced by incorporating a synergist such as piperonyl butoxide (to inhibit metabolic degradation) into the formulation. Pyrethrins and pyrethroids are also used as domestic insecticides, and pyrethroids have been widely used in public health applications for the control of insect disease vectors (Barlow, Sullivan, and Lines, 2001; Zaim, Aitio, and Nakashima, 2000). Occupational exposure is discussed in Chapter 15.

The very low volatility of the pyrethroids and pyrethrins (<1 mPa, and in some cases <1 nPa, at 20°C) means that inhalation of vapour is an insignificant route of exposure, and so inhalation of dust or aerosol droplets, ingestion, or dermal

Pyrethrin I

Cypermethrin
(cyano group is absent in permethrin)
(dichloro group is dibromo in deltamethrin)
(dichloro group is dimethyl in cyphenothrin)

Fenvalerate

Figure 4.1 Structures of pyrethrin I and some common pyrethroids, emphasizing their structural similarities

Table 4.1 Classification[a] and toxicity[b] of some pyrethrins and pyrethroids

Type I	LD_{50}	Type I/II	LD_{50}	Type II	LD_{50}
Bifenthrin	55	Cyphenothrin	420	Cyfluthrin	400
Bioallethrin	1030	Fenproponate	28	Cyhalothrin	144
Bioresmethrin	8000	Flucythrinate	67	Cypermethrin	900
Kadethrin	650			Deltamethrin	52
Permethrin[c]	430–9000			Fenvalerate	450
Pyrethrins[d]	340–900				
Tetramethrin	> 5000				

[a]Based on poisoning syndrome seen in the rat.
[b]Acute oral LD_{50} in female rats.
[c]Varies with isomeric composition.
[d]Complex mixture of active components.

absorption represent the major routes of human exposure. The blood half-life of pyrethroids is of the order of tens of hours (Anadon *et al.*, 1996; Gray *et al.*, 1980), and some investigators have found even shorter half-lives. Thus cyfluthrin has a plasma half life of 19–86 min in man (Leng and Lewalter, 1999). Inherent toxic potential can be high, as intravenous LD_{50}s range from >250 down to 0.5 mg/kg (Ray, 1991), but toxicity is limited in practice by rapid hydrolysis in blood and liver. Hence exposure routes that allow rapid absorption (such as droplet inhalation) show the greatest toxicity, while slower routes (such as dermal) show much less toxicity due to slow absorption through the skin and local metabolic destruction of pyrethroids in the skin (Bast, Taeschner, and Kampffmeyer, 1997). In man the bioavailability of dermal pyrethroids is about 1 per cent (Woollen *et al.*, 1992), compared with 36 per cent for gastric absorption. Hence the dermal route of exposure presents relatively little risk of systemic poisoning although, in cases of very severe skin contamination, intoxication has lasted for several weeks (He *et al.*, 1989), possibly due to a reservoir of pyrethroid bound to the epidermis. Once absorbed, pyrethroids rapidly distribute through the body, their high lipophilicity and lack of exclusion by the multi-drug transporter glycoprotein (Bain and LeBlanc, 1996) ensuring ready entry into the brain (Gray *et al.*, 1980).

Mechanisms of toxicity

The actions in pests and in non-target organisms are similar: the molecular targets present in insects being analogous to those seen in mammals. However, the relative resistance of mammals to pyrethroids is due to a combination of their faster metabolic disposal, higher body temperature, and a lower sensitivity of the target sites (Song and Narahashi, 1996b). This extensive metabolism of pyrethroids leads to some degree of oxidative stress via glutathione depletion (Giray, Gurbay, and Hincal, 2001) that may be of significance at very high exposure levels. The toxicology of the pyrethroids has been reviewed by several authors (Aldridge, 1990; Narahashi *et al.*, 1998; Soderlund and Knipple, 1995; Vijverberg and van Bercken, 1990), and a risk assessment has been published by Barlow, Sullivan, and Lines (2001). Many different mechanisms of action have been proposed for the pyrethroids, which are potent agents with a wide range of actions on biological systems (Table 4.2), but only those effects that are seen at the lower concentrations are likely to have much biological significance. Unfortunately, much of the published data on pyrethroid concentrations must be used with caution, since pyrethroids have very low water solubilities; readily partition into lipids; and show potent binding to glass and plastics. This means that it is very difficult to be certain of the true concentration (Ray, Sutharsan, and Forshaw, 1997), or to compare directly across systems with different physical–chemical characteristics. Furthermore, while actions at some of these target sites have appreciable biological consequences at less

Table 4.2 Some potential targets of pyrethroids in mammals

Target	Concentration[a]	Reference
Protein phosphorylation	10^{-13} M	Enan and Matsumura (1993)
Voltage-gated sodium channels	10^{-10} M	Ghiasuddin and Soderlund (1985)
Voltage-gated chloride channels	10^{-10} M	Ray *et al.* (1997)
Noradrenaline release	10^{-10} M	Brooks and Clark (1987)
Membrane depolarization	10^{-8} M	Eells *et al.* (1992); Rekling and Theophilidis (1995)
Voltage-gated calcium channels	$<10^{-7}$ M	Hagiwara *et al.* (1988)
GABA-gated chloride channels	10^{-7} M	Lawrence *et al.* (1985)
Nicotinic receptors	10^{-7} M	Sherby *et al.* (1986)
Mitochondrial complex I	10^{-7} M	Gassner *et al.* (1997)
Lymphocyte proliferation	10^{-6} M	Diel *et al.* (1998)
Mitochondrial ATP-ase	10^{-5} M	Prasada Rao *et al.* (1984)
Intercellular gap junctions	10^{-5} M	Hemming *et al.* (1993); Tateno *et al.* (1993)
Chromosomal damage	10^{-4} M	Barrueco *et al.* (1992)

[a]Nominal pyrethroid concentration needed to produce a significant effect in the system used.

than 1 per cent modification (Song and Narahashi, 1996b), others may require 90 per cent modification before an effect is seen. Hence, in constructing Table 4.1 the lowest concentration needed to produce a significant biological response has been cited, since the ED_{50} can underestimate potency.

Pyrethroids are primarily functional toxins (Narahashi *et al.*, 1998), readily causing hyper-excitation, but having little or no direct cytotoxic potential in mammalian cells. Thus, unexcitable mammalian cells are little affected: a number of pyrethroids only producing growth inhibition at 10^{-5} M, and no cytotoxicity at 10^{-4} M (Squiban, Marano, and Ronot, 1986). In contrast, the interaction with the sodium channel shows dissociation constants of the order of 10^{-8} M (Soderlund, 1985), producing profound hyper-excitation in nerve or muscle cells. Similarly, hepatocytes in culture showed decreased viability after treatment with permethrin only at 100 ng/10^6 cells (El Tawil and AbdelRahman, 1997), a level which is approximately 10–100 times higher than that reached in the brain of intoxicated animals. Obviously such high concentration *in vitro* studies are of very limited relevance to human toxicology.

The nervous system is the primary target, and studies of genotoxicity and carcinogenicity have either proved negative or indicated little toxic potential (Nehez, Lorencz, and Desi, 2000). Pyrethroids do not have significant oestrogen receptor actions (Sumida *et al.*, 2001). Chronic toxicity studies have generally shown only reactive changes associated with the increased xenobiotic metabolic load represented by the pyrethroids. High dose chronic feeding studies in rats and dogs with fenvalerate have produced a foreign body response in liver following deposition of cholesterol ester crystals (Kaneko *et al.*, 1988) but this does not represent a hazard

at lower doses. Just subtoxic levels of type II pyrethroids have a tumour promoter-like activity in rat liver, although not acting as tumour initiators (Hemming, Flodstrom, and Warngard, 1993). This action has been attributed to an inhibition of gap-junction communication, but the high pyrethroid concentrations needed to act on gap junctions *in vitro* (Table 4.1) casts doubt on the hypothesis (Hemming, Flodstrom, and Warngard, 1993; Tateno *et al.*, 1993).

Differential toxicity of isomers

In mammals the 1*R* isomers are active and the 1*S* isomers inactive and essentially non-toxic. Isomerism at the third carbon of the cyclopropane ring gives *cis* and *trans* isomers which show insecticidal activity (Elliott *et al.*, 1978) but differential mammalian toxicity, with the *cis* isomers being about 10 times more potent than the *trans* isomers (Gray, 1985). A final chiral centre is generated if a cyano substituent is added to the alcohol, giving eight possible isomers. Again this affects potency, with only the α-*S* and not the α-*R* forms being toxic both to insects and mammals. This stereospecificity has been exploited in the synthesis of pure isomers such as deltamethrin to produce a remarkable degree of selective toxicity (Glomot, 1982). A practical consequence of this is that the toxicity of products such as permethrin, which are commonly sold as mixtures, can vary from product to product. Thus the rat oral LD_{50} value of commercial permethrin varies from 430 to 8900 mg/kg (WHO/IPCS, 1990) and is largely determined by the *cis* isomer content.

Actions at sodium channels

Voltage gated sodium channels are vital to the function of most excitable cells, and are seen in all organisms from jellyfish upward. They are responsible for the generation of the inward sodium current that produces the action potential in most cells, and are closed at normal resting potentials. Their structure and functions have been reviewed by Marban, Yamagishi, and Tomaselli (1998) and by Conley and Brammar (1999). They consist of an α subunit, which confers pore function, resembles those of other voltage-gated ion channels, and can take several possible isoforms; and the β_1 and β_2 subunits, which may be absent in non-mammalian channels, and which modify the basic function of the α subunit. There are many variant forms of the α subunit, 10 being characterized in the rat, and channels are also subject to glycosylation and phosphorylation which further modify function. The channel is highly ion selective, favouring sodium over potassium ions by a factor of 30, yet maintaining a relatively high conductance of 10–25 pS. Depolarization-driven activation, via the *m*-gate of Hodgkin and Huxley (1952), is sensed by the four S4 segments in the α subunit, and probably mediated by the S6 segment. Time-dependent inactivation of the channel, via the *h*-gate, is less well defined in molecular terms, but probably is related to the cytoplasmic domain between the S3 and S4 segments of the α subunit. Unfortunately, a standardized nomenclature for

the many channel iso-forms has not been accepted, and descriptions based on pharmacological properties (e.g. tetrodotoxin resistant) or tissue source (e.g. brain I, II, III) are widely used. Expression is controlled by many different genes.

The interaction of pyrethroids with the sodium channel has the effect of slowing both the activation and inactivation properties of the sodium channel (Ginsburg and Narahashi, 1993), leading to a stable hyper-excitable state. This effect is amplified by the high level of expression of sodium channels in most excitable cells, which means that only about 0.1 per cent of sodium channels need to be modified by a pyrethroid in order for the extra current generated to render a cell hyperexcitable (Song and Narahashi, 1996b). Although activation is slowed at the single channel level, this high density of sodium channels means that sufficient unmodified channels are always present to ensure that the activation phase of the action potential is not appreciably delayed. However, in the falling phase of the action potential even the low proportion of modified channels can generate enough extra current to delay inactivation. This slower rate of inactivation of pyrethroid-modified channels generates a prolonged depolarizing 'tail' current (Figure 4.2) that follows the normal action potential. This 'tail' can trigger a second action potential if the current is large enough and lasts for more than the 0.5 ms needed for the unmodified sodium

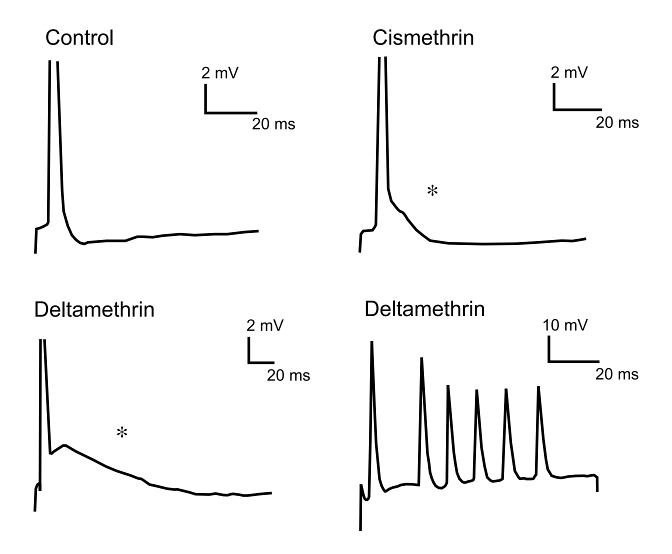


Figure 4.2 Effects of cismethrin (a type I pyrethroid) and deltamethrin (a type II pyrethroid) on rat diaphragm muscle action potentials. At the end of the unchanged action potential spike an abnormal depolarizing after potential (∗) develops, which is short after cismethrin or long after deltamethrin. When large enough, this depolarization causes a train of action potentials to follow the normal one, as in the last record (displayed at a lower voltage gain)

channels to recover excitability. Hence, what would normally be a single action potential can become converted into a train of abnormal discharges. This abnormal hyperactivity produces a profound disruption of neuronal function. Action potential amplitude generally remains constant, the effect of pyrethroids in delaying the recruitment of open channels after depolarization (which would decrease action potential amplitude), and the delaying of the closing of these same channels with time (which would increase amplitude) cancelling out in most systems (Ginsburg and Narahashi, 1993).

Another significant feature is that, after modification by pyrethroids, sodium channels retain many of their other normal functions, such as their selectivity for sodium ions and conductance. Their link with membrane potential is also retained, although shifted to increased excitability (Narahashi *et al.*, 1998). This means that after exposure to moderate doses of pyrethroids cells can continue to function – but in a new and relatively stable state of abnormal hyper-excitability. In insects this state corresponds to the incapacitating but sublethal effect known as 'knock down'. The amplitude of the sodium current continues undiminished until the level of sodium entry associated with this hyper-excitability overwhelms the capacity of the sodium pump to remove it (Narahashi, 1985). Very high concentrations of pyrethroids, or levels of hyperactivity beyond those which the cell can sustain, can thus cause depolarization and conduction block (Vijverberg and de Weille, 1985). This depolarization is more readily produced by those pyrethroids that hold the sodium channel open longest.

An important characteristic of the pyrethroid-generated tail current is that the amplitude and duration are independent. The current amplitude is dependent only on the proportion of sodium channels modified, and hence shows a sigmoid relationship with pyrethroid concentration or dose. The current duration is dependent only on the pyrethroid structure: some pyrethroids, such as permethrin, holding the channel open for relatively short times and others, such as deltamethrin, holding it open for much longer. Individual pyrethroids thus generate a characteristic time constant for prolongation of the sodium channel tail current that is virtually independent of dose (Brown and Narahashi, 1992). There is a continuous distribution of time constants across the range of pyrethroid structures, with type I pyrethroids producing the shorter time constants, and type II pyrethroids producing the longer time constants. This holds true in both insects and amphibia where the time constants vary from tens of milliseconds to seconds (Vijverberg and de Weille, 1985), and in mammals, where the absolute time constants are rather shorter (Wright, Forshaw, and Ray, 1988). In all cases the threshold of hyper-excitability is reached once the decaying tail current remains above the threshold for sufficiently long to precipitate a second abnormal action potential. This can be achieved either by increasing the amplitude *or* the duration of the tail current.

The different forms of the sodium channel show differential sensitivity to pyrethroids. Pyrethroids act most readily on the tetrodotoxin-resistant subtype of the sodium channel (Song and Narahashi, 1996a), which is expressed in the developing

mammalian brain, and in the adult dorsal root ganglia. The tetrodotoxin-resistant channels are 10 times more sensitive than tetrodotoxin-sensitive channels in the same cells (Ginsburg and Narahashi, 1993). Insect sodium channels are 100 times more sensitive than the rat brain IIa channel (Warmke *et al.*, 1997), which in part explains the resistance of mammals. The rat brain IIa form of the sodium channel is sensitive to type II, but not type I pyrethroids, an effect enhanced 20-fold by the presence of the acessory β_1 subunit (Smith and Soderlund, 1998). Channels expressing only the α subunit are capable of showing all of the characteristics of pyrethroid modification, but require relatively high pyrethroid concentrations of 10^{-8}–10^{-7} M (Trainer *et al.*, 1997). It has been proposed that some of the regional selectivity of action of the pyrethroids parallels the distribution of sensitive sodium channel subtypes, although there are at present only limited data to support this attractive idea. Thus, striatal brain slices showed neurotransmitter release in response to type II pyrethroids that was not shown by hippocampal slices of similar capacity (Eells and Dubocovich, 1988). When these brain regions were examined *in vivo*, the striatum was the first site to show EEG discharges and hyper-excitability (Ray, 1980), whereas the hippocampus showed only enhanced inhibition (Joy *et al.*, 1990). A similar differential was seen when the activation of early response genes by mild pyrethroid intoxication was examined, hippocampus showing very little response, but specific cortical, thalamic, and hypothalamic areas showing marked activation (Hassouna *et al.*, 1996). Unfortunately, the distribution of the different sodium channel subtypes across these brain regions is not yet known. Peripheral nerve (SNS/PN3) sodium channels are highly sensitive to pyrethroids, especially to type II compounds which produce effects at 10^{-9} M (Soderlund, Smith, and Lee, 2000), and action at these channels may be relevant to the production of paraesthesia. Unfortunately, all such studies are difficult to interpret at present because it has proved difficult the reproduce the high pyrethroid sensitivity of *in vivo* systems in single channel electrophysiological experiments. Hence it is not possible to rank the various sodium channel types in terms of absolute sensitivity, other than when they are co-expressed in the same test system.

Pyrethroid action on the sodium channel shows a marked stereospecificity that results in some isomers being far more toxic than others (Soderlund, 1985): the 1*R* and 1*S* *cis* isomers binding competitively to one site, and the 1*R* and 1*S* *trans* isomers binding non-competitively to another (Narahashi, 1986). The 1*S* forms do not modify the channel function, but do block the effect of the I*R* isomers.

Actions at other sites

Many target sites other than the sodium channel have been suggested to be relevant to poisoning. These are summarized in Table 4.2. To put these into context, the tissue concentration of pyrethroids in the brain during intoxication varies from 1 to 30 μmol/kg tissue (Anadon *et al.*, 1996; Rickard and Brodie, 1985) in rats, although the free concentration is probably lower. The complex nature of the effects

of pyrethroids on the central nervous system has led various workers to suggest that they also act via antagonism of γ-aminobutyric acid (GABA) mediated inhibition; modulation of nicotinic cholinergic transmission; enhancement of noradrenaline release; or direct actions on calcium or chloride ion channels. However, since neurotransmitter-specific pharmacological agents offer only poor or partial protection against poisoning, it is likely that these other effects represent only secondary mechanisms of action of the pyrethroids. Indeed, most neurotransmitter release is secondary to increased sodium entry (Eells *et al.*, 1992).

Action on the voltage-dependent chloride channel has been proposed as an additional target of type II pyrethroids (Forshaw and Ray, 1990). Voltage-sensitive chloride channels are found in brain, nerve, muscle, and salivary gland, and their function is to control cell excitability: chloride and sodium conductance having reciprocal effects on membrane excitability (Adrian and Marshall, 1976). Many functionally different kinds of chloride channels are seen, with the maxi chloride channel class (Franciolini and Petris, 1990) being sensitive to pyrethroids. Maxi channels have not been characterized at the molecular level, but are activated by depolarization, have high conductance, are calcium independent, and are inactivated by protein kinase C phosphorylation (Forshaw and Ray, 1993). Type II pyrethroids decrease chloride channel currents both *in vitro* (Ray, Sutharsan, and Forshaw, 1997), and *in vivo* (Forshaw and Ray, 1990). The pyrethroid-induced decrease in maxi chloride channel current is brought about by a fall in open state probability, which serves to increase excitability and therefore would synergize pyrethroid actions on the sodium channel. Of the pyrethroids that have been tested, only the type II affected maxi chloride channels (Ray, Sutharsan, and Forshaw, 1997). Since agents such as ivermectin and pentobarbitone, which open chloride channels, have antagonist actions on pyrethroid-evoked salivation, choreoathetosis, and repetitive firing in skeletal muscle (Forshaw, Lister, and Ray, 2000), it seems likely that chloride channel actions contribute to most components of the type II poisoning syndrome. Indeed, an action on voltage-gated chloride channels may play a major part in the generation of the salivation and myotonia, although its role in the centrally generated signs of poisoning appears to be limited to synergizing the primary action of pyrethroids on the sodium channel.

Voltage-dependent calcium channels have also been proposed as a target of pyrethroids, and are good candidates in insects, with primary effects on T-type calcium currents being seen at 10^{-7} M (Duce *et al.*, 1999). However, some mammalian calcium channels appear to be less sensitive: tetramethrin producing 75 per cent block of T and 30 per cent block of L currents in neuroblastoma cells only at 10^{-5} M, and type II pyrethroids having no effect (Narahashi, 1988). In contrast rabbit sino-atrial node cells were far more sensitive, 10^{-7} M tetramethrin (a type I pyrethroid) producing complete block of T current, but no effect on L current until 10^{-5} M (Hagiwara, Irisawa, and Kameyama, 1988). Unfortunately, the effect of lower concentrations was not reported. More sensitive actions on non-adrenaline release (Brooks and Clark, 1987), may also be mediated via calcium channels.

At relatively high concentrations pyrethroids can also act on GABA-gated chloride channels (Bloomquist, Adams, and Soderlund, 1986), which may contribute to the seizures seen with severe type II poisoning. Several other reports have suggested a role for the $GABA_A$ receptor–ionophore complex in components of type II pyrethroid toxicity. Deltamethrin inhibits binding of ligand to the mammalian GABA complex by 50 per cent at 10^{-7} M (Lawrence, Gee, and Yamamura, 1985) and GABA-stimulated chloride flux is reduced by 72 per cent by 10^{-6} M *cis*-cypermethrin (Abalis, Eldefrawi, and Eldefrawi, 1986). As with voltage-gated chloride channels, these effects are specific for type II pyrethroids. Contrary evidence against a major GABA-antagonist effect of pyrethroids is that deltamethrin does not reduce $GABA_A$-mediated hippocampal inhibition *in vivo* (Joy and Albertson, 1991), nor does the *in vivo* toxicity of deltamethrin correlate well with its potency in inhibiting GABA-induced chloride influx (Ramadan *et al.*, 1988). An even more marked differential is seen in invertebrates, with sodium effects at 10^{-12} M and GABA effects at 10^{-6} M (Chalmers, Miller, and Olsen, 1987). Pyrethroids, however, potentiate pentylenetetrazole convulsions by interaction with benzodiazepine binding sites (Devaud, Szot, and Murray, 1986) at more reasonable doses, and this may indicate a potential for action via GABA. Similarly, type II pyrethroids show dose additivity with GABA antagonists in terms of acoustic startle (Crofton and Reiter, 1987) although this does not prove a common mechanism, since deltamethrin also enhances tryptamine toxicity (Chanh *et al.*, 1984). A further problem for the GABA hypothesis is that the choreoathetosis, which is seen at lower doses than those that evoke seizures, has been shown to be of spinal origin (Bradbury *et al.*, 1983). GABA is not an important neurotransmitter in the spinal cord, and Ogata, Vogel, and Narahashi (1988) have shown that deltamethrin does not block the $GABA_A$-gated chloride current in dorsal root ganglia. Of the pharmacological agents acting on the GABA receptor, baclofen had no therapeutic properties in deltamethrin-poisoned rats (Bradbury *et al.*, 1983). GABA antagonists were ineffective at preventing the increased hippocampal inhibition produced by deltamethrin (Joy and Albertson, 1991) or in controlling deltamethrin choreoathetosis in rats (Cremer *et al.*, 1980), although diazepam did prevent the seizures associated with late stage poisoning (Gammon, Lawrence, and Casida, 1982). Diazepam was also ineffective in fenvalerate poisoned humans (He *et al.*, 1989). It therefore appears that the undoubted potential of type II pyrethroids to act at the GABA receptor is of limited practical significance other than in cases of severe intoxication.

The peripheral benzodiazepine receptor has also been proposed as a target of pyrethroids, Devaud, Szot, and Murray (1986) finding that the proconvulsant action of low doses of both type I and type II pyrethroids were blocked by the peripheral benzodiazepine receptor antagonist PK11195. This antagonist also reduced the inhibitory effect of ivermectin on deltamethrin-evoked salivation in rats (Forshaw, Lister, and Ray, 2000), suggesting that some component of the salivation is mediated via the peripheral benzodiazepine receptor. Competitive binding at both central and peripheral type benzodiazepine receptors has been shown to occur in rat parotid and submandibular glands (Yamagishi and Kawaguchi, 1998).

The mechanism whereby pyrethroids interact with ion channels is not known, but type II pyrethroids stimulate protein kinase C dependent protein phosphorylation at as low a concentration as 10^{-13} M *in vitro* by a direct mechanism (Enan and Matsumura, 1993). Since both sodium and chloride ion channel activity is modulated by phosphorylation state, this is likely to be a very important mechanism of action. Pyrethroids are also capable of acting directly in systems with no phosphorylation capacity, but at somewhat higher concentrations (Forshaw, Lister, and Ray, 1993).

Pyrethroids have no direct anticholinesterase activity (Ray and Cremer, 1979) and have little effect on acetylcholine sensitivity of muscle (Sherby *et al.*, 1986) but do inhibit acetylcholine-activated calcium flux at 10^{-7} M and slow the desensitization of the nicotinic receptor (Sherby *et al.*, 1986). This may lead to some degree of potentiation of nicotinic transmission in the central nervous system (CNS). The marked increase in neocortical blood flow seen during pyrethroid intoxication is cholinergically mediated, but this probably represents an indirect effect of pyrethroids on the cholinergic system (Lister and Ray, 1988).

A more sensitive effect is the stimulation of noradrenaline release from brain synaptosomes, which has an ED_{50} of only 2.9×10^{-9} M for deltamethrin, although type I pyrethroids were ineffective (Brooks and Clark, 1987). Cultured cells proved much less sensitive, requiring 10^{-5} M (Bickmeyer, Weinsber, and Wiegand, 1994). This noradrenaline release parallels increased calcium entry, and may indicate an action via voltage-sensitive calcium or sodium channels. Related effects are seen in whole animals, where deltamethrin markedly increases plasma noradrenaline (Cremer and Seville, 1982) and enhances the noradrenaline-mediated contraction of mesenteric blood vessels and cardiac contractility (Forshaw and Bradbury, 1983), and also in early development (see section on Developmental Neurotoxicity below). It has been suggested that the calcium effects may be mediated by an action on calmodulin, but calmodulin-stimulated phosphodiesterase activity is inhibited by pyrethroids only at 10^{-6}–10^{-4} M (Rashatwar and Matsumara, 1985). Narahashi (1986) has also described a decrease in fast calcium current in neuroblastoma cells at concentrations similar to those prolonging sodium current.

Other biochemical changes, such the marked increases in cerebellar cyclic GMP and changes in the concentration of several neurotransmitters in the brain (Aldridge *et al.*, 1978; Hudson *et al.*, 1986) which parallel type II motor symptoms, appear to be entirely secondary to the increased motor activity, and could not be reproduced in cerebellar slices (Lock and Berry, 1981).

Systemic poisoning

Fortunately there have been relatively few reports of systemic poisoning, since the use of adequate protective clothing will prevent intoxication (Moretto, 1991). However, systemic poisoning can occur under conditions of misuse or inadequate user protection (He *et al.*, 1989), and in a study of sprayers where dermal contamination was poorly

controlled, 0.3 per cent showed signs of mild pyrethroid poisoning (Chen *et al.*, 1991). When systemic toxicity does occur, the central signs of poisoning can be difficult to control, and may be confused with intoxication by other pesticides such as anticholinesterases, which also cause salivation and hyper-excitability.

Given the common formulation of pyrethroids with organic solvents, symptoms of poisoning can be complicated by solvent toxicity, and solvents may also introduce additional skin effects. Mild poisoning symptoms may also be amplified by anxiety (Lessenger, 1992) which may itself be precipitated by fear or by the disconcerting paraesthesia resulting from dermal contact with pyrethroids (Flannigan *et al.*, 1985). A study of 144 persons reporting ill-health in association with domestic use of pyrethroid insecticides and recruited following an appeal on German television, showed a variety of complaints developing several days after exposure (Müller-Mohnssen, 1999). However, there was no non-exposed comparison group, and a within-group exposure measure (pyrethroids in house dust), which confirmed pyrethroid use, showed little correlation between exposure level and symptom reporting. Hence evidence of causation must be considered weak in this study. The majority of the cases also involved the so-called 'acquired intolerance of chemicals'. In some low exposure cases poisoning may be more apparent than real. Thus, of 64 cases of self-diagnosed pyrethroid intoxication following very low level exposure to a variety of pyrethroids, 58 showed either no somatic effects or ones that were unrelated to pyrethroid exposure, and 6 showed reversible changes that were of ambiguous aetiology. No CNS or peripheral nervous system (PNS) lesions were found in any of these cases (Altenkirch *et al.*, 1996). Such cases are in marked contrast to the very clear signs of intoxication seen in occupational or suicidal poisonings (He *et al.*, 1989), and may therefore relate more to public perception of hazard than to real toxicity.

Animal studies show that all the motor signs of systemic pyrethroid intoxication are generated at the spinal level: being relatively unchanged by destruction of the brain (Bradbury *et al.*, 1983). Recording from central and peripheral sites shows that a modest level of hyperactivity in sensory fibres is amplified at the first synapse, and becomes further amplified in polysynaptic pathways (Forshaw, Lister, and Ray, 1987).

Despite the continuous variation in duration of the abnormal sodium current with pyrethroid structure described in the section Mechanisms of Toxicity, the effects of all pyrethroids can be described in terms of just two well-demarcated poisoning syndromes (see below) that are seen in both mammals and insects. The longer current prolongations are far more disruptive, and pyrethroids with time constants of more than about 10 ms (the normal time constant of the unmodified sodium channel being about 0.5 ms) causing uncoordination, choreoathetosis, seizures, and direct effects on skeletal and cardiac muscle and salivary gland. Reflex hyperexcitability can be seen as a dose-dependent combination of enhancement and suppression (Hijzen and Slangen, 1988; Wright, Forshaw, and Ray, 1988). This is called the type II, or choreoathetosis/salivation, syndrome (Aldridge, 1990).

Those pyrethroids producing shorter prolongations of less than about 10 ms in mammals (Wright, Forshaw, and Ray, 1988) cause a less complex syndrome of simple reflex hyper-excitability and fine tremor, which is termed the type I, or tremor, syndrome, and closely resembles that produced by DDT (Aldridge, 1990). Pyrethroids causing intermediate time constants of about 10 ms produce a complex mixed syndrome in which the characteristic signs of both type I and type II poisoning are superimposed (Wright, Forshaw, and Ray, 1988). Almost all reports of human poisoning relate to the more potent type II pyrethroids, so it is not certain how well this description of the two syndromes applies to man, although what has been described fits quite well with experimental animal observations.

A similar division into two classes has been made by a number of authors for systemic effects in insects (Gammon, Brown, and Casida, 1981), in amphibians (Ruigt and van den Bercken, 1986), and in mammals (Barnes and Verschoyle, 1974; Verschoyle and Aldridge, 1980; Wright, Forshaw, and Ray, 1988). Some authors have objected to such a division on the grounds that qualitatively similar electrophysiological changes are produced by all active pyrethroids (Staatz-Benson and Hosko, 1986) or because some pyrethroids show intermediate characteristics (Gammon, Brown, and Casida, 1981; Verschoyle and Aldridge, 1980), but it has practical value. All type II pyrethroids have a cyano substituent, but not all type I pyrethroids lack one, the *trans* and *cis* isomers of flurocyphenothrin somewhat confusingly producing type I and II effects, respectively. Both pyrethroid classes have a similar range of mammalian toxicity but, for commercial pesticides, the type II pyrethroids such as deltamethrin and cypermethrin generally are more toxic than the type I pyrethroids such as permethrin. A classification of the more common pyrethroids is given in Table 4.1.

Both type I and II pyrethroids cause marked adrenal activation in rats, probably by a direct stimulation of noradrenaline release (Brooks and Clark, 1987), with the increases in blood adrenaline and noradrenaline accompanying motor signs (Cremer and Seville, 1982). The type II pyrethroid, deltamethrin, causes increased corticosteroid secretion at even lower doses (de Boer *et al.*, 1988). Hence even moderate pyrethroid poisoning occurs against a background of profound adrenal activation, and this has been proposed as the mechanism whereby low doses of fenvalerate reduce conditioned avoidance behaviour in rats (Moniz, Bernardi, and Spinosa, 1994).

Type I poisoning

The type I pyrethroids produce the simplest poisoning syndrome and produce sodium tail currents with relatively short time constants (Wright, Forshaw, and Ray, 1988). Poisoning closely resembles that produced by DDT and was first clearly described by Verschoyle and Aldridge (1980). It involves a progressive development of fine whole-body tremor, exaggerated startle response, uncoordinated twitching of the dorsal muscles, hyper-excitability, and death (Ray, 1982b). The tremor can be so severe as to

double whole-body metabolic rate in the rat at sublethal dose levels (Cremer and Seville, 1982), and can lead to prostration and death. At sublethal dose levels respiration and blood pressure are well sustained (Ray, 1982b) but plasma noradrenaline, lactate, and to a lesser extent adrenaline, are greatly increased (Cremer and Seville, 1982). Type I effects are generated largely by action on the CNS, as shown by the good correlation between brain levels of cismethrin and tremor (White *et al.*, 1976) and the induction of tremor by small quantities of cismethrin directly injected into the CNS (Gray and Rickard, 1982; Staatz, Bloom, and Lech, 1982). Poisoning is associated with marked increases in both spinal (Carlton, 1977; Staatz-Benson and Hosko, 1986) and brain stem excitability (Forshaw and Ray, 1986), although not with marked effects on higher centres. Thus, lethal doses of cismethrin do not induce cortical EEG spiking (Ray, 1982a) although supralethal doses in paralysed, ventilated animals do (Staatz and Hosko, 1985). Also, when cismethrin is injected into the lateral ventricles tremor is seen only when enough is given to reach the brain stem (Grey and Rickard, 1982) and, although primary increases in reflex excitability are seen in the brain stem and spinal cord, only secondary effects are seen at the cerebellar, thalamic, and cerebral cortical levels (Ray, 1982a). There is also evidence for repetitive firing in peripheral sensory nerves (Forshaw and Ray, 1986; Staatz-Benson and Hosko, 1986; Wright, Forshaw, and Ray, 1988). Such repetitive firing is analogous to that seen in amphibians (Vijverberg, Ruigi, and van den Bercken, 1982) and probably contributes to the hyper-excitable state produced by the central actions of the type I pyrethroids.

Type II poisoning

The type II pyrethroids produce a more complex poisoning syndrome and act on a wider range of tissues. They give sodium tail currents with relatively long time constants (Wright, Forshaw, and Ray, 1988), which may be the reason for their ability to act on the whole range of excitable tissues. Human type II poisoning seems to be characterized by paraesthesia (if via the dermal route), dizziness, nausea, listlessness, and muscular fasciculations (Chen *et al.*, 1991). More severe poisoning caused epigastric pain, nausea and vomiting (if via the oral route), hypersalivation and pulmonary oedema, opisthotonos, seizures, and coma (He *et al.*, 1989).

First described by Barnes and Verschoyle (1974), type II poisoning in rats involves progressive development of nosing and exaggerated jaw opening similar to that seen in response to an irritant placed on the tongue; salivation, which may be profuse; increasing extensor tone in the hind limbs causing a rolling gait; uncoordination progressing to a very coarse tremor; choreoform movements of the limbs and tail often precipitated by sensory stimuli; generalized choreoathetosis (writhing spasms); tonic seizures; apnoea; and death (Ray, 1982b). At lower doses more subtle repetitive behaviour is seen (Brodie and Aldridge, 1982) and learned behaviour is impaired (Moniz, Bernardi, and Spinosa, 1994). In dogs, similar symptoms are seen but salivation and upper airway hypersecretion and gastrointestinal symptoms are more prominent (Thiebault, Bost, and Foulhoux, 1985). Unlike the type I syndrome, type II pyrethroids generally

decrease rather than increase the startle response to sound (Crofton and Reiter, 1984), although this is a complex response and at low doses some type II pyrethroids give an increased startle (Hijzen and Slangen, 1988). The cerebral cortical response to sound is also depressed (Ray, 1980) and the latency of the visual response increased (Dyer, 1985). As in type I poisoning, plasma noradrenaline is increased by type II pyrethroids, but there is also a large increase in adrenaline and in blood glucose (Cremer and Seville, 1982; Ray and Cremer, 1979) which is not seen in type I poisoning. Type II, but not type I pyrethroids also increase skeletal and cardiac muscle contractility (Forshaw and Bradbury, 1983; Forshaw, Lister, and Ray, 1987), but these effects are limited by physiological compensation which maintains blood pressure at normal levels. As with type I pyrethroids, the primary action is on the CNS, since symptoms correlate well with brain concentrations (Rickard and Brodie, 1985) and can be reproduced in part by microinjection into the CNS (Brodie, 1985; Staatz, Bloom, and Lech, 1982). The former injection studies showed, however, that actions at all levels of the neuroaxis are needed to reproduce the full range of effects. Thus, although choreoathetosis can be reproduced in spinal rats (Bradbury *et al.*, 1983), other symptoms are generated at higher levels and are associated with EEG spiking at cortical and subcortical sites which ultimately progresses to slow-wave activity and loss of consciousness not seen for type I pyrethroids (Condes Lara, Graff Guerrero, and Vega Riveroll, 1999; Ray, 1980). By this stage secondary involvement of many neurotransmitter systems is seen, since both specific and non-specific pharmacological interventions can control the seizures.

Although there is evidence for increased neuronal activity in both the spinal cord (Staatz-Benson and Hosko, 1986) and brain (Ray, 1980; Staatz and Hosko, 1985), the type II pyrethroids do not produce the repetitive activity in sensory nerves seen after type I pyrethroids (Wright, Forshaw, and Ray, 1988). This again is analogous to the effects seen in amphibians (Vijverberg, Ruigi, and van den Bercken, 1982). As might be expected, both classes of pyrethroid produce large increases in brain glucose utilization, this being most marked in sensory/motor areas (Cremer, Cunningham, and Seville, 1983; Cremer and Seville, 1985). Such increases seem to be secondary to increased neuronal activity and are paralleled by increased brain blood flow – except in the cerebral cortex, where the flow increase is disproportionally large (Lister and Ray, 1988).

Mixed/intermediate poisoning

Complex mixed signs representing a combination of types I and II poisoning are produced by some pyrethroids. These appear to represent a true superimposition of the type I and type II poisoning syndromes (Wright, Forshaw, and Ray, 1988) and to represent a transitional state. Evidence in support of this is given by measurement of the time constants of the sodium after-potential produced by the pyrethroids. These are short for the type I, and long for type II pyrethroids. For the whole range of pyrethroids, time constants range from 5 to 1772 ms in amphibians (Vijverberg

and de Weille, 1985) and 2.3 to 33 ms in mammals (Wright, Forshaw, and Ray, 1988). The structures producing mixed signs fit in the middle of these ranges.

The related question of what might happen in the case of simultaneous exposure to a type I and a type II pyrethroid appears not to have been addressed in the whole animal. However, Song *et al.* (1996) used tetramethrin (type I pyrethroid) and fenvalerate (type II pyrethroid), and compared their actions on tetrodotoxin-resistant sodium channels in isolated neonatal rat dorsal root ganglion cells. The measure used was the degree of prolongation of the time constant, which reflects the class of the pyrethroid effect and is more or less independent of dose. If tetramethrin was added to preparations showing a characteristic fenvalerate response, this disappeared and was replaced by a response very similar to that generated by tetramethrin alone. When the preparation was washed, it reverted to a fenvalerate response (the tetramethrin effect being readily reversible in this system). Although these data should be interpreted with some caution since both pyrethroids were used at very high nominal concentrations, the result implies that type I and II actions may be mutually exclusive.

Pathology

The major toxic hazard presented by pyrethroids to adults is acute excitation. However, near lethal doses of all classes of pyrethroids can give rise to an axonal degeneration in peripheral nerve resembling Wallerian degeneration (Aldridge, 1990; Calore *et al.*, 2000; Rose and Dewar, 1983), but this effect is reversible within 7 days, and is only seen at dose levels that produce prolonged and severe motor signs. Central neuropathology has been described in one study of adult rats given 15 daily doses of the type II pyrethroid deltamethrin at doses just below the threshold for motor signs (Husain, Malaviya, and Seth, 1994). The animals were reported to show 40 per cent increases in the weight of pons/medulla and hippocampus apparently without morphological change, and also degeneration of cerebellar Purkinje cells. These findings do not appear to be internally consistent, and their significance must be considered questionable.

No central pathology was found in rats after sustained tremor evoked by a type I pyrethroid (Holton *et al.*, 1997). However, Wu and Liu (2000) described severe neuronal loss in the hippocampus and in cortical areas of rats given a single 12.5 mg/kg intraperitoneal dose of the type II pyrethroid deltamethrin. This neuronal death involved both necrosis and activation of markers of apoptotic death. The dose was reported to produce motor signs of neurotoxicity but ‘much less death’ than higher doses. Unfortunately this same pattern of cell death would also be expected from hypoxia consequent on seizures, and it is not clear how much (if any) might have been due to a direct effect of deltamethrin. A histopathological survey of brain from rats given 20 mg/kg deltamethrin orally (half of the LD_{50} for that route) found no such changes (D. E. Ray and A. W. Brown, unpublished data).

Paraesthesia and local irritation

In addition to systemic toxicity, sufficiently high doses of pyrethroids can produce important local effects: skin contamination producing paraesthesia (Wilks, 2000); ingestion producing gastrointestinal irritation (Thiebault, Bost, and Foulhoux, 1985); and inhalation producing upper respiratory tract irritation (Pauluhn and Machemer, 1998). The gastrointestinal irritation is rare (being limited to cases of ingestion) and has not been well studied, but presumably is a similar phenomenon to the more common dermal paraesthesia. Respiratory tract irritation can be produced at comparable thresholds in rats and man, but is rarely reported. Dermal exposures far below the threshold for systemic poisoning can lead to a local paraesthesia (Wilks, 2000), which is evoked by all classes (the pyrethrins and types I and II pyrethroids), with a severity roughly in proportion to their systemic toxic potential (Aldridge, 1990). Although the plant products associated with impure pyrethrum extracts can give rise to classical contact dermatitis, the pure synthetic pyrethroids produce only a simple paraesthesia, not inflammation or erythema (Flannigan *et al.*, 1985). However, inflammation can be evoked by some pesticide solvents or by scratching. Two cases of a probable immune reaction in man have been described, one a reaction to a flumethrin sheep dip (Box and Lee, 1996), and another case of severe eczema following exposure to a bioresmethrin/pyrethrin spray (Müller-Mohnssen, 1999). Such reactions are very rare, however, considering the widespread use of pyrethroids. Paraesthesia is not idiosyncratic however, being dose-dependent in severity and duration, and lasting for 4–30 h after a single application. When mild, the sensation is of continuous tingling or pricking or, when more severe, burning. The effect is annoying but not disabling and does not appear to be associated with any lasting ill-effects on nerve conduction (LeQuesne, Maxwell, and Butterworth, 1980). An animal model of paraesthesia is available (Cagen *et al.*, 1984; McKillop *et al.*, 1987). In addition, an electrophysiological test (enhancement of the supernormal phase in two pulse stimulations) can detect peripheral nerve hyper-excitability up to 24 h after exposure in animals (Parkin and LeQuesne, 1982). This test has also proved useful for monitoring pyrethroid effects in man (He *et al.*, 1991).

The mechanism of paraesthesia has not been studied directly, but presumably the sensation is caused by abnormal pyrethroid-induced repetitive activity in skin nerve terminals which are exposed to higher concentrations than penetrate the rest of the body. Such an idea is supported by the observation that another sodium channel opening agent, veratrine, produces a similar response to the pyrethroids in a guinea pig model of paraesthesia (McKillop *et al.*, 1987). Repetitive activity in sensory nerves is certainly seen during systemic intoxication in rats (Wright, Forshaw, and Ray, 1988) but the effect is much less than that seen in the CNS, suggesting that skin receptors are not especially sensitive to pyrethroids. The reversible peripheral nerve vacuolation produced by pyrethroids is an unrelated effect, since it appears only when severe systemic poisoning is produced (Aldridge, 1990).

Developmental neurotoxicity

Neonatal rats are 4–17 times more vulnerable than adults to the acutely toxic doses of types I and II pyrethroids, probably wholly due to their lesser capacity for metabolic detoxification (Cantalamessa, 1993), an observation that is consistent with neonates and adults having similar brain concentrations at different, but equi-toxic doses (Sheets *et al.*, 1994). At lower dose levels producing threshold toxic effects, even neonates have sufficient pyrethroid-metabolizing capacity, and there is no evidence to suggest that neonatal rats are more susceptible to low level acute toxicity than adults (Sheets, 2000).

A number of more specific effects of exposure to pyrethroids during early development have been described in rats or mice. Cypermethrin at 4 per cent of the LD_{50} over postnatal days 10–16 caused an increase in renal D_1 receptor density in rats which persisted at least until day 9 (Cantalamessa *et al.*, 1998). A similar dose given to pregnant rats on days 7–16 of gestation (which produced no overt maternal toxicity or change in pup birth weight) elevated plasma adrenaline and noradrenaline in the pups (Santoni *et al.*, 1999). This effect was detectable between postnatal days 15 and 90, and was associated with a concomitant increase in circulating T lymphocytes and a decrease of numbers in the spleen. The authors proposed that these immune effects were secondary to increased noradrenaline release in the spleen, and it is of interest that these surprisingly long-lasting effects were seen at a relatively low dose level.

The pyrethroids permethrin and deltamethrin have also been reported to induce reproducible and permanent changes in behaviour and neurochemistry of adult mice similar to those of DDT when administered directly to the neonate in a long series of studies using the same protocol (Eriksson, Ahlbom, and Fredriksson, 1992; Eriksson, Archer, and Fredriksson, 1990; Eriksson and Fredriksson, 1991; Eriksson and Talts, 2000; Talts, Fredriksson, and Eriksson, 1998). These effects were seen at dose levels that are not acutely toxic. It should be noted that at much higher doses DDT acts directly on sodium channels in the same way as type I pyrethroids (Woolley, 1982). It was proposed that the pyrethroid effects resulted from exposure at a critical period of rapid brain growth, during which the developing motor and sensory systems are believed to be especially vulnerable to chemical insult (Eriksson, Ahlbom, and Fredriksson, 1992; Eriksson, Archer, and Fredriksson, 1990). Neonates are known to be sensitive to neuroactive pharmaceuticals such as haloperidol, postnatal exposure to which causes similarly enduring changes in dopamine receptor mediated behaviour which persists into adulthood (Cuomo, Cattabeni, and Racagni, 1983; Rosengarten and Friedhoff, 1979; Thiel *et al.*, 1989). In the case of pharmaceuticals though, the dose required is close to that producing acute effects in the adult. It has been proposed that the decrease in muscarinic receptor density, and a decrease in open field habituation produced by neonatal treatment of mice with very low doses of bioallethrin and

deltamethrin is a result of an induced lack of appropriate cholinergic inhibitory capacity in the neonate (Eriksson, Ahlbom, and Fredriksson, 1992). However, the acute increases in receptor density (4 or 7 per cent of control) produced by DDT or type I pyrethroids (Ahlbom, Fredriksson, and Eriksson, 1994; Eriksson, Ahlbom, and Fredriksson, 1992) appear to be too small to account for any subsequent maldevelopment, given the large receptor reserve shown by many receptors (Bencherif *et al.*, 1995; Ek and Antonsson, 1993; Zhu, 1993) unless the changes were concentrated in a specific subpopulation. These positive results also contrast with a lack of effect of longer term, higher dose dietary administration of pyrethroids in rat multi-generation studies conducted for regulatory purposes (Deshmukh, 1992; Gomes, Bernardi, and Spinosa, 1991), except where the dose level so high as to produce maternal toxicity (Abdelkhalik, Hanafy, and Abdelaziz, 1993). Others (Muhammed *et al.*, 2000) have reproduced the lasting change in muscarinic receptor density produced by low doses of DDT in mice, but not that produced by pyrethroids. The overall physiological significance of this pyrethroid-induced muscarinic receptor deficit clearly needs further investigation. It is interesting that a major type of sodium channel is found in the developing rat brain with a peak density level in brain stem on postnatal day 10–21 and with a higher binding affinity for saxitoxin than in the adult (Xia and Haddad, 1994). This could be a potential site for low dose pyrethroid and DDT developmental neurotoxicity. However, it appears that a clarification of the question of the reproducibility and applicability of the effects seen in mice to other species will be needed before any more general conclusions can be drawn with regard to the potential developmental neurotoxicity of pyrethroids.

A different effect is the delayed development of the blood–brain barrier in rat pups given cypermethrin at doses having no effect in adult rats (Gupta, Agarwal, and Shukla, 1999). The minimum dose needed to produce this effect was only 2 per cent of the adult LD_{50}, but represented 20 per cent of the neonatal LD_{50} (Cantalamessa, 1993), so it is possible that the effect was relatively non-specific. Vascular damage and delayed neuronal development was also reported in neonatal rats given 0.7 $mg\,kg^{-1}\,day^{-1}$ deltamethrin i.p. for 5 days (Patro *et al.*, 1997). This dose produced a marked decrease in pup body weight though, so it is likely in this case also that the developmental delay was non-specific in origin.

Reference values for synthetic pyrethroids

Reference values for synthetic pyrethoids have been set for intake of residues from food (acceptable daily intakes and in some cases acute reference doses) and for operator exposure. Unlike organophosphates, where the critical effect in animal studies is almost always acetylcholinesterase depression, the effects defining the critical no adverse effect levels on which these reference doses are based are very variable (Table 4.3). Although neurotoxicity is sometimes seen, the critical adverse

Table 4.3 Reference doses for some synthetic pyrethroids

Name	ADI (mg/kg bw)	Effects seen in critical study or studies	Species	Reference
Bioresmethrin	0–0.03	Hepatotoxicity	Rats (2-year study)	FAO/WHO (1992)
Cyfluthrin	0–0.02	Decreased weight gain	Rat (2-year study)	FAO/WHO (1997a)
Cypermethrin	0–0.05	Decreased weight gain, blood and liver changes; reduced litter size and weight in reproduction studies	Rat (90 d, 2-year, reproductive toxicity studies)	FAO/WHO (1996)
Alpha-cypermethrin	0–0.02	Skin irritation, clinical signs including tremors	Dog (1 year)	FAO/WHO (1996)
Deltamethrin	0–0.01	Behavioural changes; chewing, abnormal gait and tremor; liquid faeces.	Dog (1-year and 2-year studies)	FAO/WHO (2001)
		Behavioural changes, hepatotoxicity	Rat (2-year study)	
Flumethrin	0–0.004	Skin lesions and reduced body-weight gain in the parents and reduced pup survival[a]	Rat (multi-generation study)	FAO/WHO (1997b)
Permethrin	0–0.05	Clinical signs (tremor),[b] changes in body and organ weights (ovary); increased blood glucose	Rat (2-year study)	FAO/WHO (2000)
Pyrethrins	0–0.04	Decreased weight gain; tumours in the liver, thyroid, and skin	Rat (2-year study)	FAO/WHO (2000)

[a]Possible behavioural changes in the pups; cramped posture, hypothermia, vocalization.
[b]Varies with isomeric composition.

effects in other cases are non-specific such as failure of weight gain or, if organo-specific, not obviously related to the insecticidal action of this group of compounds. However, dermal paraesthesia and the related local gastrointestinal effects may explain some of the non-specific end points.

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5 Toxicology of Miscellaneous Insecticides

Roland Solecki

Introduction

This chapter covers miscellaneous widely used insecticides, which include both synthetic products and formulations of natural origin. Long before the advent of synthetic insecticides, materials of natural origin provided the means for controlling insects affecting the human population both directly and indirectly. Insecticides of natural origin include pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. For example, the use of plant extracts (e.g. azadirachtin, rotenone) or sex pheromones [e.g. furanone, (Z)-9-tricosene] has insecticidal applications.

It is accepted that plant ingredients may exhibit toxic or other undesirable side-effects on non-target organisms, including humans. Thus, nicotine was frequently more toxic to mammals than to the insect pests. However, mostly plant insecticides are inherently less harmful than conventional pesticides. They usually affect only the target and closely related organisms, in contrast to broad spectrum, conventional insecticides that may also affect organisms as different from insects as birds and mammals. However, there is a great pitfall in the popular view that substances of natural origin are harmless. Therefore, any material of natural origin, which is produced to introduce as a pest control agent, should be subjected to the same searching examination for potential toxicity to man as is applied to synthetic pesticides (WHO, 1967).

Insecticides of natural origin have certain disadvantages in that they are often mixtures of active and inactive components and the active ingredient content may be low, depending on origin, harvest, storage conditions, and manufacturing process. Contamination of plant products with mycotoxins or other hazardous substances may occur. The biological variability may result in different toxicological properties of different batches. This in turn makes the toxicological characterization of the active substances difficult and very often less reproducible than is the case with single synthetic active ingredients. Thus, an essential prerequisite for toxicological evaluation is that identity and quality are subject to constant and strict control. To some extent, in recent decades, naturally based substances such as

Table 5.1 ADIs or chronic reference doses derived for miscellaneous synthetic insecticides

Active substance	ADI (mg/kg bw)	Safety factor	NOAEL ($mg\,kg^{-1}\,day^{-1}$)	Relevant study/species	Reference
Abamectin	0.002	50	0.12	Multigen/rat	JMPR (1997)
Spinosad	0.02	100	2.68	2-year/dog	JMPR (2001a)
Imidacloprid	0.057	100	5.7	24-month/rat	JMPR (2001b)
Fipronil	0.0002	100	0.025	104-week/rat	JMPR (2000)
Indoxacarb	0.02	100	2.0	24-month/rat	US-EPA (1997b)
Diflubenzuron	0.02	100	2.0	52-week/dog	JMPR (1985)
Tebufenozide	0.02	100	1.8	1-yr/dog	JMPR (1996)
Methoprene	0.1	100	12.5	90-day/dog	JMPR (1987)

extracts from plants and microorganisms have been produced in larger amounts with more consistent technical specifications than previously. Recently, the most successful insecticides based on naturally occurring substances are synthetic analogues of biological neurotoxins and growth regulators. Table 5.1 summarizes the active substances of the synthetic insecticides discussed in this chapter and their acceptable daily intakes (ADIs). Where available, acute reference doses (ARfDs) are also mentioned for these insecticides and in Table 5.2.

Table 5.2 Acute Reference Doses (ARfD) derived for miscellaneous synthetic insectivides

Active substance	ADI (mg/kg bw)	Safety factor	NOAEL ($mg\,kg^{-1}\,day^{-1}$)	Relevant study/species	Reference
Spinosad	not allocated	not necessary	–	absence of acute toxic alerts	JMPR (2001a)
Imidacloprid	0.4	100	42	acute neurotoxicity/ rat	JMPR (2001b)
Fipronil	0.003	100	0.3	repeated dose neurotoxicity/ rat	JMPR (1997b)
Indoxacarb	0.02	100	2.0	24-month/rat	US-EPA (1997b)
Diflubenzuron	not allocated	not necessary	–	absence of acute toxic alerts	JMPR (2001c)
Tebufenozide	0.05	100	5	2-wk/dog	JMPR (2001d)
Methoprene	not allocated	not necessary	–	absence of acute toxic alerts	JMPR (1987)

Neuroactive insecticides

The neuroactive insecticides covered in this section are based on antibiotics, bacterial fermentation products, nicotine-derived substances, and the phenylpyrazole and oxadiazine families.

Avermectins (abamectin, ivermectin)

Some antibiotics used in medicine are also applied as pesticides to control not only bacteria but also fungi, mites, and insects. The avermectins discovered in the 1970s are derived from the soil bacterium *Streptomyces avermitilis*. These complex macrocyclic disaccharides have shown biological activity against insects, nematodes, and arthropods by potentiation of GABAergic neuronal and neuromuscular transmission. The hyperpolarization of neuronal membranes mediates paralysis in arthropods and nematodes.

Abamectin (Figure 5.1) was developed for agricultural use. It is composed of 80 per cent avermectin B1a and 20 per cent avermectin B1b. The structural difference between B1a and B1b is that B1a has a C_2H_5-group and the B1b has a CH_3-group attached to one of the ring structures. When avermectin B1 is applied to plants, a plant photodegradation product is formed, which is not present in animals. This delta-8,9-isomer possesses avermectin B1-like toxicological activity. Further photodegradation of avermectin B1, which results in polar degradates in plants but not in animals, does not possess avermectin B1-like toxicological properties.

(i) R = CH_3

(ii) R = H

Figure 5.1 Chemical structure of abamectin: 5-*O*-demethylavermectin A_{1a} (i) mixture with 5-*O*-demethyl-25-de(1-methylpropyl)-25-(1-methylethyl)avermectin A_{1a} (ii)

The related substance, ivermectin, although not used as a pesticide, is discussed in view of its structure and toxicological similarity to abamectin. Ivermectin was introduced into human medicine mainly for use against *Onchocerca volvulus* and also into veterinary medicine against animal parasites. Several toxicity studies suggest that abamectin and ivermectin have a comparable order of toxicity in mice, rats, rabbits, and dogs.

Absorption, distribution, excretion, and metabolism

Avermectins are well absorbed dermally, but only moderately well after oral administration. The parent compound accounted for up to 50 per cent of the total radioactivity in tissues. Abamectin is distributed throughout all major tissues with highest concentrations in fat, followed by kidneys and liver. It is excreted after enterohepatic circulation, mainly via faeces. The animals eliminated approximately 70–80 per cent in faeces over 7 days. Only 1 per cent was excreted via urine. Only a small proportion of absorbed avermectins is metabolized in mammals (Anonymous, 2000). The metabolic pathways are mainly via demethylation and hydroxylation. As main metabolites, *O*-desmethyl-, hydroxymethyl- and delta-8-9-isomer-moieties were identified. In an *in vivo* study on rhesus monkeys, the dermal penetration was estimated to be less than 1 per cent of the applied dose.

Animal toxicity

The acute oral toxicity is high with LD_{50} values from 1.5 mg/kg bw in rats to 85 mg/kg bw in the Mallard duck with mainly neurotoxic symptoms. The dermal LD_{50} was $>$300 mg/kg bw in rats and $>$3000 mg/kg bw in rabbits. Abamectin is not irritant or sensitizing. The repeated dose toxicity is characterized by decreased body weight gain and effects, mostly acute, on the central nervous system (CNS) such as tremors, ataxia, and mydriasis. Body weight gain and the CNS is also the main target in rats and mice after long-term exposure and there is a steep dose–response relationship.

No evidence of oncogenic potential has been found in rats and mice.

The reproductive toxicity of avermectins has been investigated in several two-generation studies on rats. The critical adverse effects were those on pups during early lactation including increased retinal folds in weanlings, increase of dead pups at birth, decreased viability and lactation indices, and reduced pup body weight at parental toxic dose levels including reduced body weight gain or neurotoxic signs, e.g. tremors. Teratogenicity studies in rats, mice, and rabbits have shown increases of malformations mostly at maternally-toxic dose levels, which, particularly in rats, were not clearly dose-related. Some mice strains showed cleft palate and exencephaly after application of abamectin and its delta-8-9-isomer with CF-1 mice being extremely sensitive. The CF-1 mice strain is sensitive to orally administered ivermectin in comparison with other species, e.g. rabbits, rats, or dogs. This high

sensitivity is considered to be associated with a deficiency in the expression of P-glycoprotein, which results in a marked increase in the compounds in brain and plasma. It has been shown that the tissue distribution of the 8,9-isomer as well as the oral LD_{50}, the subacute effect, and the neurotoxicity appears to be related to the CF-1 mice genotype for P-glycoprotein expression (Lankas, Cartwright, and Umbenhauer, 1997). Data on the effects of the 8,9-isomer indicate a strong relationship between an increase in cleft palate and reduced P-glycoprotein expression (Wise, Lankas, and Umbenhauer, 1997). Because of the species-specific P-glycoprotein expression and great heterogenicity of CF-1 mice this strain is considered to be an inappropriate model for studying the toxicity of avermectins (JMPR, 1997a).

Abamectin can be considered to be a specifically neurotoxic substance via opening of GABA-controlled chloride channels (Anonymous, 2000).

Genotoxicity

Avermectins exhibit no genotoxic or mutagenic potential.

Human toxicity

In humans, more than 50 000 000 doses of ivermectin have been administered for treatment of parasitic diseases, with no report of toxicity directly attributable to the drug. The main effects noted in humans infected with *Onchocerca* spec. and treated with ivermectin have been those arising from the death of the parasites, the so-called Mazzotti reaction characterized by arthralgia, pruritus, fever, hypertension, tachycardia, headache, and ocular changes (JECFA, 1992). The only direct adverse effect was a minor hypersensitivity seen in some patients. The very limited data on reproductive toxicity in humans indicate that ivermectin does not increase the incidence of birth defects. The adverse effects experienced by the small number of persons accidentally exposed to ivermectin by self-injection or oral ingestion included pain at the injection site, variable blood pressure, nausea, paraesthesia, urticaria, or mydriasis, vomiting, tachycardia, and somnolence. Since commercially available abamectin formulations of pesticides contain only a low amount of the active ingredient, severe intoxications are only possible if relatively large quantities of the formulation are ingested, generally with suicidal intention.

Reference doses

The no observable adverse effect level (NOAEL) of 0.12 mg/kg bw from the multi-generation study in rats was used for setting the JMPR ADI. Because of the hyper-susceptibility of rats post-natally, a safety factor of only 50 was applied. The ADI of 0.002 mg/kg bw was supported by the NOAEL of 0.24 mg/kg bw from the one-year dog study (JMPR, 1997a). No ARfD has been set.

Spinosyn products (spinosad)

Spinosad (Figure 5.2) is a natural fermentation product produced by the actinomycete bacterium, *Saccharopolyspora spinosa*. It has insecticidal activity and has a structure consisting of a large complex hydrophobic ring, a basic amine group, and two sugar moieties. Spinosad is composed of numerous spinosyns, but nearly all of the insecticidal activity of spinosad is produced by the two closely related compounds spinosyns A and D, in a ratio of approximately 7:1. These two spinosyns differ from each other only in the substitution of hydrogen by a methyl group and represent about 88 per cent of the composition of spinosad. The remaining components in spinosad consist of a number of additional spinosyns, which have other minor substitutions at various locations in the molecule, and impurities consisting of inorganic salts, carbohydrates, and proteinaceous materials that may be expected from the fermentation process.

Insects exposed to spinosad exhibit classical symptoms of neurotoxicity, including lack of coordination, prostration, tremors, and other involuntary muscle contractions leading to paralysis and death. Although the mode of action of spinosad is not fully understood, it appears to affect nicotinic and GABA receptor function. Spinosad has proven effective in controlling many chewing insect pests with such

Spinosyn A: R = H

Spinosyn D: R = CH_3

Figure 5.2 Chemical formula of spinosad: mixture of spinosyn A {2-[(6-deoxy-2,3,4-tri-*O*-methyl-a-L-mannopyranosyl) oxyl-13-[(5-dimethylamino) tetrahydro-6-methyl-2H-pyran-2-yl I oxyl-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-14-methyl-1H-*as*-Indaceno (3,2-dl oxacyclododec in-7,15-dione, [2R-[2R*,3aS*,SaR*,5bS*,9S*,13S* (2R*,5S*,6R*)14R*,16aS*,16,bR*11(9Cl)} and Spinosyn D {2-((6-deoxy-2,3,4-tri-o-methyl-a-L-mannopyranosyl)oxyl-13-[5-(dimethylamino)tetrahydro-6-methyl-2H-pyran-2-ylloxyl-9-ethyl-2, 3, 3a, Sa, Sb, 6, 9, 10, 11, 12, 13,″-4,16a,16b-tetradecahydro-4,14-dimethyl-1H-*as*-Indaceno (3,2-dl oxacyclododecin-7,5-dione, (2S-2R*,3aS*,5aR*, 5bR*, 9R*,13R*(2S*, 5R*,6S*)14S*,16aR*,16bR*l](9ci)})

high selectivity that more than 70 per cent of beneficial insects and predatory wasps are left unharmed.

Absorption, distribution, excretion, and metabolism

Following oral gavage, 80 per cent of spinosyn A and 66 per cent of spinosyn D were rapidly absorbed. Peak blood concentrations of radiolabel were achieved 1–6 h after administration. The residues were initially widely distributed with highest residues in perirenal fat, liver, kidneys, and lymph nodes. In the thyroid gland, a slow rate of decline was observed. This resulted in higher concentrations in the thyroid than in other tissues, where the decline was more rapid. However, the absolute tissue levels were very low. Ninety per cent of the administered radiolabel was readily excreted within 48 h after single oral dose application. Spinosad is excreted primarily in the faeces. Most of the faecal radioactivity originates from biliary excretion. Urine and faecal excretion was almost complete at 48 h after/dosing. The routes and rates of excretion were not greatly affected by repeated administration. Spinosad is extensively metabolized, primarily via *O*-demethylation and/or glutathione conjugation. There are no major differences in the bioavailability, routes or rates or excretion, or metabolism of spinosyn A or spinosyn D following oral administration in rats.

A rat *in vivo* study has shown a dermal absorption of approximately 1 per cent. The comparison of the *in vitro* studies in rats and humans has indicated a 2-fold higher penetration in rats for the concentrate, but a 10-fold higher penetration for the dilution.

Animal toxicity

Spinosad is of low acute toxicity after oral or dermal administration and by inhalation. The oral LD_{50} is >2000 mg/kg bw for rats and mice. The dermal LD_{50} is >5000 mg/kg bw for rabbits and the inhalation LC_{50} is >5.18 mg/L air for male and female rats. Spinosad is mildly irritating to eyes. It is not irritating to the skin of rabbits and non-sensitizing to the skin of guinea pigs.

The main histopathological effects associated with repeated exposure to spinosad in all test species were cellular vacuolation, inflammatory changes including necrosis, histiocytosis, and regenerative and degenerative changes in a wide range of tissues. Electron microscopy of selected tissues from rats and mice has shown that the cytoplasm of affected cells contained clear vacuoles that consisted of variable numbers of secondary lysosomes, which contained concentric cytoplasmic lamellar inclusion bodies, reflecting a lysosomal storage disorder. While such disorders may arise through a variety of mechanisms, which prevent degradation of cell constituents that are usually processed in the lysosomes, spinosad probably acts through a largely physicochemical mechanism associated with its cationic amphophilic structure (JMPR, 2001a). The vacuolation is largely reversible on withdrawal of treatment. A number of other effects have been seen in subchronic studies on rats and

dogs, including decreases in body weights and food consumption, increased spleen, thyroid, and liver weights, altered haematology and clinical chemistry parameters, resulting in microcytic hypochromic anaemia and increased serum activity alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, and creatinine phosphokinase.

In carcinogenicity studies in mice and rats, histological effects consisted of vacuolation, particularly in the thyroid and kidneys, degeneration, and inflammatory lesions. However, there was no treatment-related increase in the incidence of neoplasms in any tissue. In a two-generation study, decreased body weights, vacuolation of the thyroid gland, and degenerative or inflammatory lesions in other tissues were observed in parental animals. Litter size and pup body weights were decreased, but only at parentally toxic dose levels. In developmental studies, spinosad caused decreased body weights in maternal rats and rabbits, but this was not accompanied by embryo-toxicity, fetal toxicity, or teratogenicity.

Spinosad did not cause specific neurotoxicity in rats in acute, subchronic or chronic toxicity studies (USEPA, 1997a).

A comparison of spinosad, spinosyn A, and spinosyn D revealed notable differences in the toxicological profiles. Whereas the toxicological effects of spinosyn A were closely similar to those of spinosad, spinosyn D failed to produce most of the haematological and clinical chemical alterations seen with spinosad or spinosyn A.

Genotoxicity

None of the genotoxicity studies showed mutagenic activity associated with spinosad.

Effects in humans

No poisonings or adverse reactions in humans have been reported.

Reference doses

The most sensitive overall toxicological end-point was thyroid vacuolation in rats treated in the diet in a 2-year study. Based on the NOAEL of 2.4 mg/kg bw, an ADI of 0.02 mg/kg bw can be derived applying a 100-fold safety factor (JMPR, 2001a). Because of the low acute toxicity and the absence of toxicological alerts in repeated-dose studies an ARfD was considered unnecessary.

Chloronicotinyl insecticides (imidacloprid)

The insecticidal properties of nicotine, extracted from the tobacco plant *Nicotiana tabacum*, have been known for many years (Buczacki and Harris, 1981). However, because of its high mammalian toxicity and relatively low insecticidal activity nicotine itself has not been widely used as a pesticide since the 1940s. Beginning

Figure 5.3 Chemical formula of imidacloprid: 1-(6-chloro-3-pyridylmethyl)-N-nitro-imidazolidin-2-ylideneamine

with nithiazine in 1979 some related synthetic substances reacting at the same receptor as nicotine were discovered. Imidacloprid (Figure 5.3) was introduced in 1992 as a new neonicotinoid insecticide. It has structural similarities to nicotine and a comparable mode of action.

Imidacloprid is active against sucking insects because of the unique plant-systemic and translaminar properties of imidacloprid. Imidacloprid poisoning in the American cockroach, *Periplaneta americana*, is characterized in sequence by a loss of leg strength, leg tremors followed by body shaking, and death (Sone *et al.*, 1994). Neurophysiological studies have confirmed that imidacloprid is an agonist at the post-synaptic nicotinic acetylcholine receptor of insects. It appears that imidacloprid acts in a way that is comparable to the action of acetylcholine on specific nerve cells. The favourable selective toxicity of imidacloprid to insects versus mammals is attributed to differences in their binding affinity or potency in the nicotinic acetylcholine receptor (Chao and Casida, 1997). It appears that this binding-site specificity for imidacloprid depends in large part on major structural differences in the neuronal nicotinic acetylcholine receptor binding sites of mammals and insects. This selectivity of the chloronicotinyl compounds for insects as opposed to mammals can be partly explained by differences in the ionization of the pyrolidine nitrogen. Imidacloprid is poorly ionized in the neutral media, in contrast to nicotine, and thus passes easily through insect lipophilic barriers (Rose, Hodgson, and Roe, 1999). The results in four aquatic arthropods suggest that imidacloprid is a selective insecticide that can be used with reasonable environmental safety toward non-target aquatic crustaceans (Song, Stark, and Brown, 1997). There was a greater correlation of susceptibility to imidacloprid within taxonomically close organisms than between organisms that share habitats.

Absorption, distribution, excretion, and metabolism

Imidacloprid is rapidly and almost completely (>92 per cent) absorbed in rats from the gastrointestinal tract following oral administration. Peak plasma concentrations are reached within approximately 3 h. The radioactivity is rapidly distributed from the intravascular space to the peripheral tissues and organs. Highest levels are observed in liver, kidney, and lungs. Elimination from the organism is fast and complete and there is no indication of any bioaccumulation potential.

Approximately three-quarters of the administered radioactivity is excreted with the urine (70–80 per cent), the remainder is found in the faeces (Anonymous, 2000). Most of the faecal radioactivity originates from biliary excretion.

The metabolism of imidacloprid in rats is rapid, and the amount of unchanged parent compound represents between 10 and 16 per cent of the dose administered. The main renal metabolites are 6-chloronicotinic acid and its glycine conjugate as well as the two corresponding imidazolidine rings containing biotransformation products.

There are no data for dermal absorption presented, so a default value of 10 per cent might be assumed for regulatory purposes.

Animal toxicity

Imidacloprid exhibits moderately acute oral toxicity to mammals with LD_{50} values of approximately 450 and 150 mg/kg bw in rats and mice, respectively. Following acute oral intake, behavioural, respiratory, and movement disturbances were observed. These symptoms were reversible within approximately 1 week. All clinical signs and neurobehavioural effects observed in an acute neurotoxicity screening study in rats were ascribed to acute cholinergic toxicity. The compound was found to exhibit virtually no acute dermal toxicity and low acute inhalation toxicity after exposure to dust. Imidacloprid exhibits no irritant properties to the skin or eyes of rabbits. No skin sensitization was observed on guinea pigs.

Reduced body weight gain was a sensitive toxicological parameter in most of the short- and long-term feeding studies in rats, mice, rabbits, and dogs. In some studies the lower body weights were coupled with a simultaneous increase in food intake. The liver was the main target organ in repeated dose toxicity studies in rats. The spectrum of changes observed ranged from induction of hepatic microsomal enzymes through disturbances of hepatic function to histologically apparent damage to the organ. The initial sign of an effect on the liver was increased activity of cytochrome P450 enzymes, accompanied by slight hepatocellular hypertrophy and necrosis, swollen cell nuclei, round-cell infiltration, and increased liver weight. Changes in blood cholesterol, triglyceride, protein, and albumin concentrations as well as increased activities of alanine aminotransferase, and alkaline phosphatase in plasma, were also observed.

The reproductive toxicity of imidacloprid was investigated in a two-generation study in rats and in developmental toxicity studies in rats and rabbits. Reduced body weight gain was the most sensitive parameter in parents and pups. An increased incidence of wavy ribs was the only developmental effect established in rats but this was related to definite maternal toxicity. Reduced body weights and increased incidences of retarded ossification of the fetuses were found in rabbits; the data showed no primary reproductive toxicity and no teratogenic potential.

In an acute neurotoxicity screening study in rats, the clinical signs and neurobehavioural effects are typical of acute cholinergic toxicity with complete recovery within 7 days following treatment. The effects observed in a subchronic neurotoxicity study in rats were related to the general toxicity of this compound.

Genotoxicity

Imidacloprid has been tested *in vitro* for point-mutagenic activity, for chromosome aberration, and for DNA repair. All these mutagenicity tests were negative. Very weak indications of sister chromatid exchange (SCE) induction were found *in vitro*, but not *in vivo*. Thus, it can be concluded, that imidacloprid exhibits no genotoxic potential (JMPR, 2001b).

Effects in humans

No adverse health effects have been reported for employees or operators and workers exposed to imidacloprid. No epidemiological studies of the effects of imidacloprid and no information on symptoms of poisoning or clinical signs were available. A 4-year-old child who ingested about 10 mg/kg bw of a veterinary preparation of imidacloprid showed no signs of poisoning or adverse health effects.

Reference doses

The NOAEL of 5.7 mg/kg bw from the chronic toxicity/carcinogenicity study in rats, which proved to be the most sensitive species, can be used for setting the ADI. Applying a safety factor of 100 results in an ADI of 0.06 mg/kg bw (JMPR, 2001b). An ARfD of 0.4 mg/kg bw was established on the basis of the NOAEL of 42 mg/kg bw in the study of acute neurotoxicity in rats.

Phenylpyrazole insecticides (fipronil)

Fipronil (Figure 5.4) is an insecticide belonging to the phenylpyrazole family. It was discovered in 1987 and first authorized for agricultural and non-agricultural use in 1993. The major advantages include a broad spectrum of activity, lack of cross-resistance with existing commercial products, and selectivity against pest species. Furthermore, fipronil is a bird repellent.

The mechanism of action for this insecticide appears to be the blockage of the GABA-regulated chloride channel. Fipronil acts as an insect-specific antagonist altering chloride ion flux, resulting in disruption of CNS activity and death of target

Figure 5.4 Chemical code of fipronil: 5-amino-[2,6-dichloro-4-(trifluoromethyl)-phenyl]-4-[(1R,S)-(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile

insects. Insect specificity relative to mammals is the result of differences in the binding affinity of fipronil to chloride channel membranes (Rose, Hodgson, and Roe, 1999). Although fipronil is selectively toxic to insects, some of the toxicity observed in mammals also appears to involve interference with the normal functioning of the GABA receptor (JMPR, 1997b).

Absorption, distribution, excretion, and metabolism

The amount of material absorbed orally appeared to be dose-dependent being about 50 per cent after low doses and about 30 per cent after high dose administration. Residues were highest in fat, with moderate levels in a variety of organs including liver and muscle. The residue levels were greater after repeated doses than single doses. The elimination half-life was relatively long. Fipronil was readily metabolized. Most radioactivity was eliminated via the faeces depending on the dose regimen, but the presence of at least 11 metabolites in faeces suggests that biliary excretion was important. Up to 14 metabolites were determined in urine. The two major urinary components were indicated to be pyrazole ring-opened compounds retaining two nitrogen atoms and the nitrile ligand from that ring. All the identified metabolites were probably eliminated as *N*-glucuronides in the urine. No significant levels of radioactivity were detected in the CO_2 trap fluids, for rats and mice.

In an *in vivo* rat study, the dermal absorption was estimated at less than 1 per cent. *In vitro*, the absorption was comparable for rats, rabbits, and humans only at low concentrations. At higher concentrations rat and rabbit skin have shown a greater penetration than human skin preparations.

Animal toxicity

Fipronil was toxic to rats and mice after oral administration of single doses with an LD_{50} of about 90 mg/kg bw/day, respectively, and after single exposure to rats by inhalation with an LC_{50} of 0.68 mg/L air. After single dermal exposures, fipronil was non-toxic to rats with an $LD_{50} > 2000$ mg/kg bw/day, but it was moderately toxic to rabbits when tested percutaneously for 24 h with an LD_{50} of 354 mg/kg bw/day. Clinical signs observed in the acute studies in rats and rabbits are consistent with effects that might be anticipated for a chemical interacting at a neurotransmitter receptor (e.g. abnormal gait, piloerection, lethargy, tremors, convulsions). Fipronil is not a dermal sensitizer in guinea pigs and not considered a skin or eye irritant. In subchronic and chronic dog studies, loss of appetite, decreased body weight gain and food consumption, and neurotoxic effects, including severe convulsions and a variety of other neurological disturbances, were observed. In mice, the liver was the main target organ with hepatocellular hypertrophy and vacuolation. Subchronic and chronic studies in rats indicated that the liver and the thyroid gland were the main target organs. This was evidenced by the presence of increased organ weights in both organs, hypertrophy and hyperplasia of the follicular epithelium of the thyroid gland, and hepato-

cellular hypertrophy. The effects on the thyroid were observed only in rats and not in mice or dogs. These species differences may be due to differences in the half-life of thyroid hormones and differences in the responsiveness of thyroid cells to thyroid-stimulating hormone (TSH). It was considered that fipronil produced a dose-related effect on the pituitary/thyroid axis: TSH was elevated and thyroxine (T4) was decreased. Triiodthyronine (T3) was significantly less affected. This disturbance appears to be related to an increase in the biliary clearance of T4 rather than a direct effect on the thyroid (e.g. an inhibition of the synthesis of T4 or T3). These imbalances in hormonal levels resulted in macroscopic and microscopic changes in the thyroid.

In a carcinogenicity study in rats, increased incidences of follicular cell adenomas and carcinomas of the thyroid gland were observed. The absence of tumours in all other tissues and the non-genotoxic profile of the compound indicate that the thyroid tumours induced by fipronil are likely to occur via a threshold mechanism. The rat is known to be very sensitive to compounds affecting thyroid hormone balance. This sensitivity of the rat to the induction of thyroid lesions compared with the relative insensitivity noted in other species indicates that these lesions have little, if any, relevance to human risk assessment. There was no evidence of carcinogenicity in mice.

In a two-generation reproduction study, mortality, convulsions, and reductions in body weight gain and food consumption were observed in adults. At necropsy, increases in liver and thyroid weights were observed. Decreased pup weights, a decrease in the mean number of live pups/litter, and convulsions were reported when the pups were beginning to consume treated diet. Therefore, these effects appear to be more related to the systemic toxicity of fipronil than a specific reproductive toxic effect. No evidence of foetal toxicity or teratogenicity was observed following oral administration of fipronil to rats or rabbits during gestation. In neurotoxicity studies with fipronil, clinical signs of toxicity consistent with the interaction of this molecule at a neurotransmitter receptor were observed. These signs were noted in all species tested. They were completely reversible in neurobehavioural tests in rats. Fipronil did not induce any microscopic lesions in the nervous system. Developmental neurotoxicity was observed post-natally in pups, including delayed swimming behaviour and increased motor activity, abnormal auditory startle response, and impaired learning and memory (JMPR, 1997b).

The mammalian metabolites of fipronil tested toxicologically have indicated acute toxicity comparable with or less than fipronil in rats. Numerous studies with fipronil-desulfinyl, one of the photodegradation products of fipronil not occurring in animals, have shown comparable toxicity, particularly in long-term studies based on clinical signs of neurotoxicity. Fipronil-desulfinyl exhibits no evidence of genotoxicity or of carcinogenicity (JMPR, 2000).

Genotoxicity

The genotoxicity studies with fipronil showed no mutagenic potential.

Effects in humans

No cases of adverse health effects in humans have been reported for fipronil.

Reference doses

Based on the NOAEL of 0.025 mg/kg bw from a 104-week study in rats and the application of a 100-fold safety factor an ADI of 0.0002 mg/kg bw for fipronil and fipronil-desulfinyl was derived (JMPR, 2000). An ARfD of 0.003 mg/kg bw for fipronil and fipronil-desulfinyl was derived on the basis of the NOAEL of 0.3 mg/kg bw in a study of neurotoxicity in rats given repeated doses of fipronil, and a safety factor of 100 (JMPR, 1997b).

Oxadiazine insecticides (indoxacarb)

Indoxacarb (Figure 5.5) is an oxadiazine insecticide composed of an insecticidally active and an insecticidally inactive isomer. The active ingredient of formulations may contain the respective isomers either in the ratio of approximately 3:1 or as a racemic (1:1) mixture. Results of toxicity studies demonstrate that the toxic effects of these compounds are related to their respective concentrations of the insecticidally active isomer.

Indoxacarb acts through blockage of the sodium channels, which is a unique mode of insecticidal action so that cross-resistance to existing insecticides is unlikely (USEPA, 1997b). Indoxacarb formulations are effective primarily on lepidopteran insects. On the other hand, they have shown good field safety to bees and other beneficial arthropods.

Absorption, distribution, excretion, and metabolism

At low doses the oral absorption of indoxacarb is approximately 75 per cent within 48 h. The radioactivity is widely distributed with highest concentrations in fat, blood, liver, and kidney. The majority of the absorbed dose is excreted within 7

Figure 5.5 Chemical formula of indoxacarb: methyl(S)-7-chloro-2,5-dihydro-2-[[(methoxycarbonyl) [4-(trifluromethoxy)phenyl] amino] carbonyl]-indeno [1, 2-e] [1, 3, 4]oxadiazine-4a(3H)-carboxylate

days (approximately 40 per cent via urine and approximately 40 per cent via faeces). Indoxacarb is extensively metabolized. The metabolism of indoxacarb can involve cleavage of the parent compound and production of aniline metabolites, which probably produce the haematological effects seen across all species tested (see below). A dermal absorption of 10 per cent in rats appears to be appropriate based on a comparison of oral and dermal toxicity studies. Because an *in vitro* study demonstrated that the absorption of indoxacarb through human epidermis is 10–12-fold less than through rat epidermis, a dermal absorption in humans of approximately 1 per cent can be assumed.

Animal toxicology

Results of acute toxicity studies in rats indicate that indoxacarb is of slight to moderate oral toxicity with an LD_{50} of 268 mg/kg bw in females and 1730 mg/kg bw in males. The acute dermal and inhalative toxicity is very low with an $LD_{50} > 5000$ mg/kg bw and an LC_{50} of 4.2 mg/L air, respectively. Indoxacarb was shown to be a skin sensitizer in the guinea pig, but was non-irritant to the skin or the eyes.

Indoxacarb causes haemolysis. In subchronic studies in rats and dogs, changes in circulating red blood cell parameters (e.g. decreases in number of erythrocytes, haemoglobin concentration, and haematocrit) were considered the most sensitive parameters to assess the haemolytic effects. A number of microscopic changes, including increased iron storage and extramedullary haemopoesis in the spleen, were also observed. Mice were less sensitive to haematological effects than rats and dogs. Chronic studies conducted in rats and mice have shown similar effects to those seen in the subchronic studies.

Indoxacarb is not oncogenic in the rat or mouse.

In the reproduction study, reductions in body weight and food consumption were observed in parental animals, but no reproductive and fertility effects were noted at the highest dose tested. In the developmental toxicity studies, reduced pup viability or retarded ossification was reported at parental toxic doses in rats or rabbits, respectively.

In an acute neurotoxicity study, some evidence of decreased motor activity, including reduced forelimb grip strength and decreased foot splay, were present.

Genotoxicity

The battery of genetic toxicity studies, including a mouse *in vivo* micronucleus study, was negative.

Effects in humans

No detrimental effects on health in manufacturing personnel have been reported for indoxacarb.

Reference doses

Based on an overall NOAEL of 2 mg/kg bw from subchronic, chronic, and neurotoxicity studies and an application of a 100-fold safety factor, both an acute and a chronic reference dose of 0.02 mg/kg bw was derived (USEPA, 1997b).

Insect growth regulators

The insecticides covered in this section act on structures or systems found in insects but not in mammals. They include the chitin synthesis inhibitors, ecdysone agonists, and juvenile hormone analogues.

Chitin synthesis inhibitors (diflubenzuron)

Diflubenzuron (Figure 5.6) is an acaricide and insecticide used as an insect growth regulator to control the larvae of insects feeding on agricultural, forest, and ornamental plants. Diflubenzuron is used primarily on cattle, citrus fruit, cotton, mushrooms, ornamentals, standing water, forestry trees, and in programmes to control mosquito larvae and gypsy moth populations.

This insecticide is a chitin synthesis inhibitor and inhibits the growth of many leaf-eating larvae, mosquito larvae, aquatic midges, rust mites, boll weevils, and flies. Diflubenzuron appears also to have an ovicidal action in disturbing chitin storage in the cuticle. Teflubenzuron is another haloaromatic substituted urea insecticide with an analogous insecticidal mode of action and similar toxicological properties.

Absorption, distribution, metabolism, and excretion

The proportion of diflubenzuron absorbed after oral administration fell with increasing dose, from about 40 per cent to 4 per cent at low and high dose levels, respectively. The highest levels of radioactivity were observed in the erythrocytes and the liver. Very little bioaccumulation in tissues was observed. The half-life of radioactivity in blood was about 14 h and over 98 per cent of the administered radioactivity had been excreted by the seventh day. Absorbed diflubenzuron was excreted primarily in urine, with involvement of biliary excretion and enterohepatic

Figure 5.6 Chemical formula of diflubenzuron: 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea

circulation. Radioactivity in expired air was negligible. Studies in rats of the metabolism of diflubenzuron showed inconsistent results. In the faeces, only unchanged parent compound was detected. The results of urinary analyses indicated that absorbed diflubenzuron is metabolized extensively before excretion in the urine and approximately 10 urinary metabolites were identified, including *p*-chloroaniline (PCA) and *p*-chlorophenylurea (CPU), which together accounted for about 2 per cent of the administered dose. The main metabolic pathway was hydroxylation of the anilino ring, cleavage of the ureido bridge, and conjugation, mainly to form the sulphate.

In an *in vivo* rat study, the dermal absorption was estimated at less than 1 per cent.

Animal toxicology

Diflubenzuron was not irritant or sensitizing and of very low acute oral, dermal, and inhalative toxicity in rats with an oral $LD_{50} > 5000$ mg/kg bw, a dermal $LD_{50} > 2000$ mg/kg bw, and an LC_{50} of > 2.5 mg/L air, respectively. Methemoglobinaemia is a notable acute effect of diflubenzuron, but acute oral studies in rats and mice with a plant protection product containing 25 per cent of the active substance indicated only marginal effects on methemoglobin levels at a dose level of 10 g/kg.

In medium- and long-term studies on mice, rats, and dogs the most sensitive endpoint was increased concentrations of methaemoglobin and sulf-haemoglobin in males and females. There were decreased erythrocyte counts and haemoglobin, Heinz bodies, increased reticulocytes, and elevated spleen and liver weights. Histopathological examinations indicated dose-related increases of haemosiderosis and congestion of the spleen, haemosiderosis and chronic inflammation of the liver, and sometimes erythroid hyperplasia of the bone marrow. Methaemoglobin formation associated with haematologic disorders was also found following dermal administration and in inhalation studies.

Based on carcinogenicity studies in rats and mice, the evidence suggests that diflubenzuron is unlikely to be carcinogenic in humans, but *p*-chloroaniline, a metabolite of diflubenzuron, is a probable human carcinogen. In a 24-month carcinogenicity study on rats, *p*-chloroaniline induced mild haemolytic anaemia, dose-related increases in methaemoglobin, non-neoplastic histopathological changes in spleen, liver, adrenals, and bone marrow, as well as a treatment-related increase in uncommon sarcomas of the spleen, which was only seen in male rats. Increased incidences of hepatocellular adenomas and carcinomas as well as haemangiosarcomas in liver and spleen were found in male mice after *p*-chloroaniline treatment in a 24-month carcinogenicity study. Also *p*-chlorophenylurea, a metabolite of diflubenzuron that is closely related to *p*-chloroaniline but where there are no adequate carcinogenicity data, is considered as having the same carcinogenic potential.

In a two-generation reproduction study, increased methaemoglobin levels, haemolytic anaemia, and signs of erythrocyte destruction were observed, including pathological effects in the spleen and liver. No effects on reproductive performance were

observed in F0 or F1 males or females. Litter and mean pup weights decreased slightly from birth to 21 days post-partum in F1 offspring at parental toxic dose levels. In the developmental toxicity studies on rats and rabbits, no maternal toxicity or toxicity to the developing fetus were observed at the limit dose level of 1000 mg/kg bw.

Diflubenzuron did not cause specific neurotoxicity in acute and repeated-dose toxicity studies.

Genotoxicity

The genotoxicity of diflubenzuron was investigated in a battery of tests *in vitro* and *in vivo*. Negative results were obtained in all the studies.

Human studies

Methaemoglobinaemia is a potential human risk from chloroaniline formed hydrolytically, but no reports of this form of toxicity have been reported in humans from diflubenzuron exposure (Reigart and Roberts, 1999). Furthermore, routine medical monitoring of workers in the production and formulation of diflubenzuron over 25 years did not reveal any adverse effects attributable to the compound.

Reference doses

The NOAEL of 2.0 mg/kg bw from the one-year study in dogs, who proved to be the most sensitive species, was used for setting the ADI. Applying a safety factor of 100, an ADI of 0.02 mg/kg bw was derived (JMPR, 1985). Although methaemoglobinaemia is potentially an acute effect, overall the toxicological profile of diflubenzuron indicates that establishment of an acute reference dose is unnecessary (JMPR, 2001c).

Ecdysone agonists (tebufenozide)

Tebufenozide (Figure 5.7) is a non-steroidal ecdysteroid agonist belonging to a new group of insect growth regulators, which have the unique characteristic of mimicking the invertebrate molting hormone 20-hydroxyecdysone.

Figure 5.7 Chemical formula of tebufenozide: N-tert-butyl-N-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide

Tebufenozide can cause a variety of molting and behavioural effects in certain orders of insects. It mimics the activity of natural ecdysteroids by inducing a premature and lethal larval molt. Mortality associated with molting suggests that tebufenozide affects the molting process, causing the formation of an incompletely sclerotized head capsule in four aquatic arthropods and the failure of ecdysis in mosquito species. These responses resulted in lethal larval molts (Song, Stark, and Brown, 1997). As an insecticide, tebufenozide has also been reported to be safe to bees and some other beneficial insects. It is an environmentally benign compound, and is non-toxic to birds and fish. Because mammals do not molt in the way insects do, any effects in mammalian species are likely to be distinct from its intended biological target in insects.

Absorption, distribution, metabolism, and excretion

In pharmacokinetic studies, only a small percentage of the administered tebufenozide was orally absorbed (at low and high dose about 35 per cent and 4 per cent, respectively). The highest levels of radioactivity were observed in liver, fat, and kidneys. Within 24 h, more than 90 per cent of the absorbed dose was excreted in faeces and only small amounts (1–8 per cent) were excreted in the urine, with trace amounts in expired air, suggesting no potential for bioaccumulation.

The main metabolic pathway was oxidation of the parent compound and 15 metabolites were identified in faeces and urine with a percentage of about 50 per cent at low dose levels. This percentage of metabolized parent compound decreased with increasing dose levels. Most of the excreted radioactivity in faeces was parent compound, but no unchanged tebufenozide was detected in urine.

Animal toxicology

Tebufenozide is of minimal mammalian toxicity with an oral and dermal $LD_{50} > 5000$ mg/kg bw and an $LC_{50} > 4.3$ mg/L air. Tebufenozide was not irritant nor did it have sensitization potential.

In medium- and long-term studies on mice, rats, and dogs, haemolytic anaemia with compensatory increased haematopoesis and increased methaemoglobin content was observed. On histopathological examination, there was dose-related splenomegaly, extramedullary haematopoesis in the spleen, and pigment deposits in the spleen, liver, and kidney. The anaemia was reversible in the dog after a 4-week recovery period. The pathogenesis of the haemolytic anaemia has not really been clarified, but it seems that oxidative damage of the erythrocytes with methaemoglobin formation was followed by intravascular haemolysis. There was also evidence of liver toxicity.

Tebufenozide is not oncogenic in the rat or mouse.

In a two-generation reproduction study, delayed pregnancy, decreased implantations and pups per litter, and increased pup mortality occurred at parentally toxic

dose levels. No developmental toxicity or teratogenicity was found in rats and rabbits.

No treatment-related effects were observed in an acute neurotoxicity study.

Genotoxicity

No potential for genotoxicity was observed.

Effects in humans

No human poisonings or adverse reactions in exposed workers have been reported.

Reference doses

The NOAEL of 1.8 mg/kg bw from the one-year study in dogs, which proved to be the most sensitive species, was used for setting the ADI. Applying a safety factor of 100, an ADI of 0.02 mg/kg bw was derived (JMPR, 1996). Based on the haemotoxicity in the short-term studies, which can also occur after a single exposure, the NOAEL of 5 mg/kg bw per day in the 2-week study in dogs was considered as a conservative estimate for ARfD setting with a safety factor of 100, resulting in an ARfD of 0.05 mg/kg bw (JMPR, 2001d).

Juvenile hormone analogues (methoprene)

The insect juvenile hormone regulates many aspects of insect life, including insect embryogenesis, larval growth, metamorphosis, reproduction, diapause, and migration. Synthetic juvenile hormone analogues such as methoprene, hydroprene, and pyriproxyfen mimic the structure and function of the natural insect hormone and presumably bind to the insect juvenile hormone receptor (Rose *et al.*, 1999).

Methoprene (Figure 5.8) is a long chain hydrocarbon ester, which is a racemic mixture of two enantiomers (R and S in a ratio of 1:1). The insecticidal activity of the compound as a juvenile hormone analogue is restricted to the S-enantiomer.

The insecticidal analogues contain functional mimics of the juvenile hormone that are poorly metabolized or not metabolized at all by juvenile hormone enzymes. Juvenile hormone activity is tightly controlled in insects by the rate of hormone

Figure 5.8 Chemical code of methoprene: isopropyl(*E,E*)-(*RS*)-11-methoxy-3,7,11-trimethyl-dodeca-2,4-dienoate

biosynthesis and this hormone regulates and controls sensitive stages of development, particularly early embryogenesis, late larval development, and metamorphosis. Since the juvenile hormone system usually is not found outside of the family of insects, the analogues are highly active and selective against insects and considered to be relatively safe for mammals, including human beings.

Absorption, distribution, metabolism, and excretion

Methoprene is rapidly and well absorbed in rats. After a single oral dose to rats, the peak plasma concentration was reached 6 h after treatment. The highest residues were found in liver, kidneys, lungs, and fat tissue. A relatively high concentration of radiolabel was found in adrenal cortex, lachrymal glands, and adipose tissue. The level declined with a half-time of about 48 h. Nearly 40 per cent was exhaled as CO_2. The remaining dose is excreted via urine and via faeces. Most of the faecal radioactivity originates from biliary excretion.

Methoprene was readily metabolized to primary and secondary metabolites. The main metabolic pathway was ester hydrolysis and *O*-demethylation to CO_2, acetate, and propionate followed by excretion or incorporation in endogenous substances and natural products such as triglycerides, bile acids, and cholesterol found in tissues, milk, and eggs. In rats, no parent compound was found in urine and bile. Unchanged methoprene was only found in faeces as a very small portion of the administered dose.

Animal toxicology

Methoprene is of low acute toxicity after oral, dermal, and intraperitoneal administration. The oral and intraperitoneal LD_{50} values in rats were >5000 mg/kg bw and >3000 mg/kg bw, respectively. The LD_{50} after dermal administration in rabbits was >3000 mg/kg bw. The substance is not considered to be a skin or eye irritant. Methoprene is not a dermal sensitizer in guinea pigs.

Repeated dose toxicity studies in which mice, rats, and dogs were exposed to methoprene in the diet showed that the compound has little toxic potential with some effects on food intake and body weight. The liver was the main target organ in rats and dogs. Hepatotoxic effects were also observed after long-term application in rats and mice.

No evidence of an oncogenic potential was found in rats and mice.

In a three-generation test, the body weight gain was slightly decreased in the parents and offspring, and the mean number of pups in the litters of the last generation born dead was increased. In a developmental toxicity study on rabbits, abortion and a decreased number of pups per litter were observed at doses that were severely maternal toxic, but there was no evidence of a teratogenic potential. In a study of developmental toxicity in mice, no toxicologically relevant effects were observed in dams or fetuses.

Methoprene did not cause any neurotoxic effects in acute and repeated-dose toxicity studies.

Genotoxicity

Methoprene exhibits no genotoxic or mutagenic potential *in vitro* or *in vivo*.

Effects in humans

No human poisonings or adverse reactions in exposed workers have been reported (Reigart and Roberts, 1999).

Reference doses

Based on the NOAEL of 12.5 mg/kg bw from a 90-day study in dogs and the application of a 100-fold safety factor an ADI of 0.1 mg/kg bw was derived for methoprene (JMPR, 1987). Since in studies with single and repeated oral doses methoprene did not induce signs indicative of acute toxicity, an allocation of an ARfD was considered unnecessary (JMPR, 2001e).

Plant insecticides

Plant insecticides are pesticidal active substances that plants produce either naturally or after genetical modification. The following plant materials with insecticidal activity are articles of commerce: nicotine alkaloids and sulphates, rotenone, azadirachtin, helleborne, ryania, sabadilla, and pyrethrum. The toxicological characterization of insecticides derived from plant sources in this section is concentrated on rotenone and neem extracts.

Rotenone

The use of rotenone-containing plants as fish poisons has been reported since the early eighteenth century. Their use as contact and stomach insecticides is more than a century old, but the rapid photodegradation by ultraviolet light has limited the commercial use of rotenone. However, it is now widely used as an agricultural and household garden insecticide to control aphids, thrips, suckers, moths, beetles, and spider mites and to control mosquito larvae when applied to pond water (Tomlin, 1999). More than 60 plant species of the family *Leguminosae* are known to produce rotenone and other rotenoids, usually in the roots. But, most commercially used plants from which rotenone is extracted are species belonging to the genera *Derris* and *Lonchocarpus*, native to Southeast Asia and South America,

Figure 5.9 Chemical formula of rotenone: [2R-(2α,6aα,12aα)]-1,2,12,12a-tetrahydro-8,9-dimethoxy-2-(1-methylethenyl)[1]benozopyrano[3,4-b]furo[2,3-h] [1]benzopyran-6(6aH)-one

respectively. Rotenone (Figure 5.9) is a mitochondrial poison, which acts by blocking NADH oxidation. The insecticidal activity seems to be based on inhibition of mitochondrial oxidation and the critical effects in insects are related to blocked nerve conduction.

Metabolism and excretion

Rotenone is metabolized largely in the liver by hydroxylation at carbons 7 and 24. The acute toxicity of the metabolites hydroxyrotenone and rotelone I seem to be comparable with that of rotenone, whereas dihydroxrotenone and rotelone II are significantly less toxic. One mechanism of detoxification was found to be 3-*O*-demethylation. Within 24 h, approximately 20 per cent of radioactivity was excreted in urine in rats and mice, respectively (Fukami *et al.*, 1969).

Animal toxicology

The acute toxicity appears to vary considerably between species, with oral LD_{50} values ranging from 60 to 3000 mg/kg bw. Factors other than species variation, which probably influences the oral absorption and consequently also the LD_{50} values, were the concentration and particle size of rotenone in the powders derived from the various rotenone-containing plants, and the diluents used for administration of the material. Several studies indicated that the finer the particle size the more toxic the preparation, and the use of oil as a diluent also increases the toxicity. Thus, the LD_{50} in rats was estimated to be between 25 mg/kg bw and 200 mg/kg bw when dissolved in olive oil and administered as a suspension in vegetable gums, respectively. The main acute effects seen initially was respiratory stimulation followed by death from respiratory failure. When rotenone has been injected into

animals, tremors, vomiting, incoordination, convulsions, and respiratory arrest have been observed (Reigart and Roberts, 1999). Derris powder is not irritant to animals and human skin, but the dust produces intense eye irritation, accompanied by the formation of pus (Hayley, 1978). In repeated dose toxicity studies, clinical signs including increased incidence of emesis and diarrhoea, reduced mean body weights, decreased haematocrit, haemoglobin, cholesterol, total lipids, and glucose levels, as well as fatty changes in the liver and kidneys, were observed. In a long-term rat study, effects observed included reduced body weight, reduced food consumption, lower total protein and albumin levels in the blood, increased blood urea nitrogen levels, and increased incidences of adrenal gland angiectasis and haemorrhage.

Whether rotenone is carcinogenic is a matter of controversy. Some authors reported increased incidences of mammary tumours in rats, which were not supported by other investigators (Gosalvez, 1983; Marquardt *et al.*, 1999). No evidence of carcinogenic activity was observed in mice (Innes *et al.*, 1969). Recent reports have shown that rotenone inhibits spontaneously and chemically induced hepatic tumorigenesis in rodents through a decrease in hepatic focal proliferation and an increase in focal apoptosis (Isenberg and Klaunig, 2000). Besides the reversible inhibition of the microtubule assembly, respiratory inhibition by blocked electron transport is assumed to occur. Chronic exposure to rotenone in rats can also reproduce the anatomical, neurochemical, behavioural, and neuropathological features of Parkinson's disease by systemic inhibition of mitochondria complex I, which caused highly selective nigrostratial dopaminergic degeneration (Betarbet *et al.*, 2000; Giasson and Lee, 2000).

In a two-generation study on rats, litter sizes and pup weights were reduced at doses toxic to the parents, as demonstrated by reduced body weight and body weight gain. Developmental studies in rats have shown increased resorptions and reductions in foetal weights and delayed skeletal ossification, including increased incidences of extra ribs, unossified sternebrae, renal pelvic cavitation, and distended ureters at maternally severely toxic dose levels in which decreased maternal body weights were usually also present (Khera, Whalen, and Angers, 1982).

Genotoxicity

Rotenone is a potential spindle poison, comparable to colchicine. Rotenone was not mutagenic in reverse mutation and unscheduled DNA synthesis assays. In Chinese hamster ovary cells, induction of sister-chromatide exchanges and chromosome aberrations was not observed, but rotenone induced aneuploidy and polyploidy in this cells line. Rotenone induced forward mutations in the mouse lymphoma assay, increased the frequency of binucleated cells, and caused a delay in the cell division. The mutagenic effects of rotenone were considered to depend on the inhibition of both the cell respiration and on the microtubuli assembly (Barham and Brinkley, 1976a, 1976b).

Effects in humans

Neither deaths nor systemic poisonings resulting from exposure to rotenone products have been reported in relation to ordinary use over many decades. Numbness of oral mucous membranes, dermatitis, and respiratory tract irritation have been reported after inhalation of dust from the powdered derris root in occupationally exposed humans. Dermatitis and respiratory tract irritation have also been reported in occupationally exposed persons (Reigart and Roberts, 1999).

Reference doses

No chronic or acute reference doses published by any regulatory body were obtainable.

Neem tree extracts and preparations (azadirachtin, dihydroazadirachtin)

For centuries, traditional Indian medicine has made use of neem tree preparations for various therapeutic purposes (e.g. for treatment of digestive and respiratory disorders, skin diseases, diabetes, infections, as well as for dental care) and as an insecticide (e.g. in malaria control). Neem tree extracts and pure azadirachdin (AZA) formulations are also used in agriculture for the control of whitefly, leaf miners, and other pests including pear psylla (Tomlin, 1999). There is evidence that AZA interferes with the molting process via antagonism with ecdysone, a naturally occurring insect hormone (Schmutterer, 1985). This hormone system is unknown among vertebrates. The related insecticidal active ingredient, dihydroazadirachtin (DAZA), is a reduction product of the naturally occurring AZA obtained from the seed kernels of the neem tree, *Azadirachta indica A. Juss* (USEPA, 1998). DAZA is structurally similar to AZA, and the two compounds are functionally identical in their anti-pupation properties. DAZA has insect growth regulator properties. The substance is an anti-feedant/anti-molting pesticide for chitin-producing invertebrates. Toxicological information on studies in laboratory or farm animals, findings reported in humans, and poisoning incidents in human infants were summarized by Jacobson (1995). The toxicological data obtained with of the neem seed extract 'NeemAzal' (a powder containing about 34 per cent azadirachtin A) have been reviewed by Niemann and Hilbig (2000).

Animal toxicology

Formulations with DAZA and AZA have very low mammalian toxicity, as demonstrated in acute oral, dermal, and inhalation toxicity studies. When fresh crude neem oil was tested for acute oral toxicity in rats and rabbits, more pronounced toxic effects occurred, including mortality and clinical symptoms comprising decreased motor activity, stupor, tremor, convulsions, and diarrhoea (Gandhi *et al.*, 1988). Histopathological examination of rats and rabbits in this study revealed lung

lesions, whereas biochemical findings in rats suggested damage to liver function. There is little information available on the irritant and sensitization potential of neem ingredients. However, most Neem formulations were not irritant to the skin and to the eyes. Both active ingredients, DAZA and AZA, test negatively for dermal sensitization. Following subchronic feeding of the neem seed extract 'NeemAzal' in rats, food consumption and body weight were decreased. The liver, thyroid, and blood (impaired coagulation) were identified as the main targets (Niemann and Hilbig, 2000). There is no evidence of oncogenic potential (Rosenkranz and Klopman, 1995). Fertility and reproductive performance were not impaired by the administration of 'NeemAzal' over two generations in rats (Niemann and Hilbig, 2000). The available information suggests that substances of neem origin were not teratogenic to laboratory animals. In contrast, there is convincing evidence of anti-fertility effects of neem seed oil and other products obtained from the neem tree in various mammalian species, including humans (Jacobson, 1995).

Genotoxicity

DAZA and AZA proved negative in mutagenicity tests *in vitro* as well as *in vivo*.

Effects in humans

Azadirachtin causes severe dermal and gastrointestinal irritation (Reigart and Roberts, 1999). However, a lack of adverse skin effects following dermal application was described in clinical trials when formulations were used for head lice and scabies control in human infants (Knust, 1998). CNS stimulation and depression have also been seen. Case reports from India and Malaysia describe severe intoxication, including irreversible brain damage or death in children following oral intake of neem oil. Signs of poisoning comprising vomiting and seizures are followed by metabolic acidosis and coma. Autopsy revealed histopathological changes in the liver and encephalopathy (Sundaravalli, Bhaskar, and Krishnamoorthy, 1982). The clinical symptoms in children and the pathological findings those of Reye's syndrome.

Occasionally, toxic effects of extracts from neem leaves on the cardiovascular system have been reported (Sivashanmugham, Bhaskar, and Banumathi, 1984). Lower blood pressure, ventricular fibrillation, and even cardiac arrest were also reported after ingestion of large volumes of extracts from neem leaves. However, the neem oil ingredient responsible for these side-effects and the underlying mechanism of toxicity is still unknown. Human experience and animal data suggest that reproduction can be affected. There is some evidence that impairment of male fertility is a potential effect, and unintentional contraceptive and abortifacient effects might occur (Jacobson, 1995). However, no unacceptable adverse effects on human health are likely from the use of registered neem products as insecticides or repellents.

Reference doses

Based on the NOAEL of 10 mg/kg bw from a multi-generation study in rats, a carcinogenicity study in mice, and a 90 day oral toxicity study in rats, a 'daily tolerable intake' (DTA) of 0.1 mg/kg bw for neem extracts was derived in Germany with the application of a 100-fold safety factor (Schellschmidt and Dieter, 2000). Since in studies with single and repeated oral doses neem formulations did not induce signs indicative of acute toxicity, the allocation of an ARfD can be considered unnecessary.

Biochemical insecticides

Biochemical insecticides are naturally occurring substances that control pests by non-toxic mechanisms. They include substances such as insect growth regulators and insect sex pheromones that interfere with mating, as well as various scented plant extracts that attract insect pests to traps. Other plant oils are complex mixtures of substances made from lemon, orange, and anise, which give fruits and seeds their characteristic odour and taste, are also used as pesticides to repel and to kill certain insects.

Attractants

Maple lactones are used in a cockroach attractant trap placed in dark or humid indoor areas where cockroaches are usually found. 9-Dodecenyl acetate is a pheromone containing the (E) and (Z) isomers. The active ingredient is the (E)-9-dodecenyl acetate pheromone. The pheromone is intended to be used in traps, dispensers, and sprays to help control destructive moths in forests and agricultural applications. Xanthine and oxypurinol, combined in equal amounts, are used in cockroach bait stations to attract and control cockroaches in indoor environments, including homes, schools, and vehicles. Formic acid is used to control tracheal mites and aid in the suppression of mites in honeybee hives. It is formulated as a gel, and contained in a vented plastic pouch that allows slow release of formic acid vapours. Although the gel is safer to use than liquid formulations appropriate protective equipment is required to preclude potential irritation to the eyes, skin, and respiratory tract of applicators. Furanone (nuranone) is a sex pheromone produced by female Japanese beetles to attract males for mating. As a pesticide, it is used to lure male Japanese beetles into traps so that the males are not available for mating. (Z)-9-Tricosene is the sex pheromone that female houseflies release to attract males for mating. The substance is used in products such as traps and 'fly paper' strips to attract males and prevent them from mating.

Effects in humans

When attractants are used as directed on the label, such products are acceptably safe for humans (USEPA, 2001). No human poisonings or adverse reactions in exposed workers have been reported.

Repellents

Oil of citronella has been used for over 50 years to repel and to kill certain insects. It is found in many familiar insect repellent products: candles, lotions, gels, sprays, and towelette wipes for use on clothing and the human body. These products are used to repel various insects, some of which are public health pests or disease vectors, including mosquitoes, biting flies, and fleas. Jojoba oil is a vegetable oil obtained from the jojoba bean. When applied to crops, pesticide products containing the oil can control white flies. Jojoba oil products are also approved for controlling powdery mildew on grapes and ornamentals. Anise oil, bergamot oil, canola oil, castor oil, cedar wood oil, citronella oil, eucalyptus oil, lavandin oil, lemongrass oil, mustard oil, orange oil, and soybean oil are all also registered as biopesticides. These oils are used as to repel and to kill certain insects. P-Menthane-3,8-diol is a biochemical pesticide derived from eucalyptus plants. This active ingredient is used to make products that are applied to human skin and clothing for the purpose of repelling insects, such as mosquitoes. It can be used in two types of consumer repellent products: a spray and a lotion. 3-[*N*-Butyl-*N*-acetyl]-aminopropionic acid, ethyl ester can be used as an insect repellent against mosquitoes, deer ticks, body lice, and biting flies. Methylcyclohexenone (MCH) is used in forests to protect live trees from spruce beetles and Douglas fir beetles. The volatile, naturally occurring chemical acts as a beetle repellent. When small amounts of MCH are attached to dead trees, beetles are prevented from aggregating on the dead trees and from large-scale reproduction.

Effects in humans, wildlife, and the environment

When used as pesticides, these repellents do not present any known risks to humans (USEPA, 2001). No human poisonings or adverse reactions in exposed workers have been reported.

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Part II

Fungicides, Herbicides and Growth Regulators

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6 Toxicology of Fungicides

Bryan Ballantyne

Introduction

Fungicides are used to eradicate or prevent the undesirable growth of fungal microorganisms in many agricultural, horticultural, and industrial situations. Numerous substances possess antifungal activity, and their chemical structural spectrum is wide and diverse, covering both inorganic and organic substances. The Appendix (p. 282) lists all those substances used for their antifungal activity and classified according to chemical structural grouping. Different authors have differing classification systems according to chemical structure, which somewhat complicates and confuses both the presentation and the discussion of fungicides. Several classification systems based on structure appear more of a web organization than a rationalized listing. In this chapter, the most effective and frequently used fungicides are discussed, using the chemical classification system shown in Table 6.1.

In addition to classification by chemical structural grouping, fungicides can be categorized agriculturally and horticulturally according to the mode of application (use) as (a) soil application fungicides, (b) foliar fungicides which are applied to plants above the ground, and (c) dressing fungicides applied after harvesting with the aim of preventing fungus development on stored crops. Fungicides may also be described according to their general mode of action as: (a) protective fungicides inhibiting the development of fungicides by either (i) sporicidal effects or (ii) foliar effects creating an environment on the plant surface not conducive to fungal growth; (b) post-infection curative fungicides which kill the developing mycelium in the plant epidermis; and (c) post-symptomatic eradication fungicides which penetrate and kill the mycelium and new spores.

It has been noted (Phillips, 2001) that the ideal fungicide should have the following characteristics: (a) low mammalian toxicity, (b) low ecotoxicity, (c) low phytotoxicity, (d) high penetration rates for spores and mycelia, and (e) limited biodegradation on the plant surface. Many fungicides combine several of these characteristics but few approach optimum for all of them.

Table 6.1 Major chemical classes of fungicides with representative examples

Halogenated substituted monocyclic aromatics
Chlorothalonil
Tecnazine
Chloroneb
Dicloran
Hexachlorobenzene
Quintozene
Pentachlorophenol
Dinocap
Dichlorophen
Carbamic acid derivatives
(a) Dithiocarbamates
Metam-sodium
Ferbam
Thiram
Ziram
(b) Ethylene bisdithiocarbamates
Maneb
Mancozeb
Zineb
Benzimidazoles/thiabendazoles
Benomyl
Thiabendazole
Thiophanate–methyl
Carbendazim
Imazalil
Fuberidazole
Chloroalkylthiodicarboximides
Captan
Captafol
Folpet
Azoles
Cyproconazole
Diniconazole
Etridazole
Fenbuconazole
Hexaconazole
Penconazole
Terbuconazole
Triadimefon
Triadimenol

Table 6.1 (*continued*)

Morpholines	
	Dodemorph
	Fenpropiomorph
	Tridemorph
Carboxanilides/oxathiins	
	Carboxin
	Oxycarboxin
Organophosphates	
	Pyrazophos
	Tolclofos-methyl
Piperazines	
	Triforine
Metallic	
	Inorganic (see Table 6.2)
	Organometallic (see Table 6.3)
Miscellaneous	
	(a) Aliphatic aldehydes
	Acrolein
	(b) Thiocarbonate
	Sodium tetrathiocarbonate
	(c) Antibiotics
	Cycloheximide
	(d) Cinnamic acid derivatives
	Dimethomorph

In general, fungicides are of low to moderate mammalian toxicology, although they are believed to have a higher overall incidence than other pesticides to cause developmental toxicology and oncogenesis (Costa, 1997). It has, for example, been estimated that more than 80 per cent of all oncogenic risk from the use of pesticides comes from a few fungicides (NAS, 1987). However, fungicides usually are responsible for only a small proportion of pesticide-related deaths, and account for only about 5 per cent or less of human pesticide exposures reported to Poison Control Centres (Blondell, 1997; Hayes and Vaughn, 1977; Litovitz, Felberg, and Soloway, 1994). It has been noted that since fungi differ significantly in morphology and physiology from other forms of life, they may be successfully combated by compounds of low toxicity to other organisms, notably mammals (Edwards, Ferry, and Temple, 1991). However, since the mechanism of injury to pathogenic fungi may be different to that for injury to mammalian systems, it is possible that the two properties may co-exist in a given fungicide molecule.

Halogenated substituted monocyclic aromatics

This group have halogenation of various substituted aromatics rings, and include derivatives of phenols and phenates. Major fungicides in this group include chlorothalonil, tecnazine, chloroneb, dichloran, hexachlorobenzene, quintozene, pentachlorophenol, and sodium pentachlorophenate. Many of these are, or metabolized to, uncouplers of oxidative phosphorylation. This can lead to excessive heat production, hyperpyrexia, liver damage, and corneal opacities.

Chlorothalonil

Chemical identification

Class: aromatic chloronitrile
Structural formula: see Figure 6.1
Molecular weight: 265.9
Common name: chlorothalonil
IUPAC name: tetrachloroisophthalonitrile
CAS name: 2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile
Synonym: 1,3-dicyanotetrachlorobenzene
CAS no.: 1897-45-6
EEC no.: 217-588-1

Uses and mechanism of activity

Chlorothalonil is a non-systemic foliar fungicide against a wide range of crops, at application rates of 1–2.5 kg/ha as suspension concentrate, water dispersible granules, wettable powder, or fogging concentrate (Tomlin, 2000). In control of mildew in paints, it conjugates and depletes thiols from germinating fungal cells, leading to disruption of glycolysis and energy production, fungistasis, and fungicidal action.

Figure 6.1 Chlorothalonil

Toxicology

Acute toxicity

The rat peroral and rabbit percutaneous LD_{50}s are both >10 000 mg/kg. By inhalation the rat 1-h LC_{50} is >4.7 mg/L.

Primary irritation

Chlorothalonil is a mild skin irritant but a severe eye irritant in rabbits.

Sensitization potential

Patch testing of the skin of Japanese farmers showed 10–28 per cent positive for chlorothalonil, with photosensitization being a factor in some (Hathaway, Proctor, and Hughes, 1993). Severe recurrent contact dermatitis was reported for workers handling chlorothalonil wood preservative (Eilrich and Chelsky, 1991). However, the incidence of sensitizing reactions is small (Hathaway, Proctor, and Hughes, 1993).

Chronic toxicology and oncogenicity

A two-year dietary study caused forestomach tumours in female mice, attributed to local gastric mucosal irritation (Wilkerson and Killeen, 1996). Technical grade chlorothalonil was fed in the diet of rats at 5063 and 10 226 ppm for 80 weeks followed by observations for 30–31 weeks (NCI, 1978a). Adenomas and carcinomas of the renal tubular epithelium were found. In the same study, mice received 2688 or 5375 (males) and 3800 or 6000 (females) ppm. No evidence of carcinogenicity was found.

Human and occupational toxicology

Chlorothalonil causes occupational allergic contact dermatitis (Bach and Pedersen, 1980; Hathaway, Proctor, and Hughes, 1993; Johnsson *et al.*, 1983; Matsushita, Arimatsu, and Nomura, 1976).

WHO toxicity class: III
EC hazard assessment: Xn, R40

Environment and ecotoxicology

Chlorothalonil is relatively immobile in soil with K_{oc} values of 1600 (sand) to 14 000 (silt). In aquatic systems, DT_{50} values range from 8 h (aerobic) to 10 days (anaerobic). In plants, the majority of residue is parent compound with 10 per cent of the metabolite 4-hydroxy-2,5,6-trichloroisophthalonitrile. Toxicity values for

avian species include: mallard ducks: acute peroral $LD_{50} > 4640$ mg/kg; 8-day dietary $LC_{50} > 10\,000$ mg/kg. Aquatic species toxicity values include 96-h LC_{50} for rainbow trout = 47 µg/L, bluegill sunfish = 60 µg/L, channel catfish = 43 µg/L and pink shrimp = 165 µg/L. The 48-h LC_{50} for *Daphnia magna* was 70 µg/L, while for *Selenastrum capricornutam* the 120-h $EC_{50} = 0.21$ mg/L; 120-h NOEC = 0.1 mg/L. For worms, the 14-day LC_{50} was >1000 mg/kg.

Tecnazine

Chemical identification

Class: chlorinated mononitrobenzene
Structural formula: see Figure 6.2
Molecular weight: 260.9
Common name: tecnazine
IUPAC and CAS name: 1,2,4,5-tetrachloro-3-nitrobenzene
CAS no.: 117-18-0
EEC no.: 204-178-2

Uses and mechanism of activity

Tecnazene is a protective and curative fungicide, available as dispersible powder, granule, and fumigant, which is used mainly to control dry rot in seed and ware potatoes. Smoke formulations are used for control of *Botrytis* in various glasshouse crops.

Toxicology

Acute toxicity

Oral rat LD_{50} are 2047 mg/kg for males and 1256 mg/kg for females: an earlier rat LD_{50} was 7500 mg/kg (WHO, 1984a). The rat intraperitoneal LD_{50} is 3500 mg/kg (Wit, Van Esch, and van Genderen, 1960), the percutaneous LD_{50} is >2000 mg/kg (Tomlin, 2000), and the 4-h inhalation $LC_{50} > 2.74$ mg/L (Tomlin, 2000).

Figure 6.2 Tecnazine

Primary irritation

Tecnazene is not irritant to rat skin but is a mild eye irritant in the rabbit.

Sensitizing potential

Skin sensitization was reported in agricultural workers (Lupuknova, 1965).

Short-term repeated and subchronic toxicology

Mice received 0, 1344, or 13 440 mg/kg tecnazine in the diet for 31 days. The only adverse effect was growth inhibition at 13 440 mg/kg (Buttle and Dyer, 1950). Rats were fed tecnazine at dietary dosages of 0, 800, 4000, and 20 000 mg kg^{-1} day^{-1} for 10 weeks. Deaths occurred at 20 000 mg/kg, and growth reduced at 4000 mg/kg (Buttle and Dyer, 1950). Rats fed tecnazine at 2000 mg kg^{-1} day^{-1} for 10 weeks had no effects on general clinical monitors, haematology, necropsy, or hepatorenal histology (Wit, Van Esch, and van Genderen, 1960).

Chronic toxicology and oncogenicity

Beagle dogs were given tecnazine perorally in capsules at daily dosages (6 per week) of 0, 3.75, 15, 60, or 240 mg/kg for 2 years. All animals died during the first year at the high dosage (WHO, 1984a). Rats were given tecnazine in the diet at 0, 750, and 1500 mg/kg for 104 weeks. There were no effects on behaviour, mortality rates, or food consumption. During the second year a slight reduction in body weight occurred with the 1500 mg/kg group. Benign or malignant mammary gland tumors were seen in 39 control females, 48 of the 750 mg/kg group, and 50 of the 1500 mg/kg group. Mammary gland adenocarcinomas were seen in 4 control females, 5 of the 750 mg/kg females, and 8 of the 1500 mg/kg females. The findings were of borderline statistical significance (WHO, 1984a). Mice received tecnazine in the diet at 0, 750, or 1500 mg/kg for 80 weeks. The only dosage-related neoplasm was pulmonary adenoma; 8 males at 1500 mg/kg compared with 4 control males (WHO, 1984a).

Development toxicology

Tecnazine, at dosages up to 200 mg/kg, was given to CD rats and C57B116 mice over gd 7–18, and CD-1 mice over gd 7–16. There were neither embryotoxic nor teratogenic effects (Courtney, Copeland, and Robbins, 1976).

Reproductive toxicology

In a two-generation reproduction study, rats were given tecnazine in the diet at dosages of 0, 200, 800, or 3200 mg kg^{-1} day^{-1} for 12 weeks prior to mating. Maternal growth

was slightly reduced and fatty infiltration of the liver occurred at the high dosage. No adverse reproductive effects occurred at any dosage (WHO, 1984a).

Metabolism and toxicokinetics

Rabbits receiving an acute peroral dose of 0.1–3 g/animal eliminated 60–78 per cent as parent material in faeces within 3 days, and 35–38 per cent in urine. Within 48 h of receiving 1–3 g tecnazine, rabbits excreted a mercapturic acid conjugate (11 per cent). Other excreted metabolites included an ether glucuronide (12 per cent), 2,3,5,6-tetrachloroaniline (10 per cent), unconjugated 4-amine-2,3,5,6-tetrachloroaniline (2 per cent) and an ethereal sulphate (1 per cent) (Betts, James, and Thorpe, 1955; Bray *et al.*, 1953).

Human and occupational toxicology

Tecnazine is a mild skin and eye irritant. However, cases of skin sensitization have been reported in agricultural workers (Lupuknova, 1965). Exposure of the general population is mainly through food residues, which are below the FAO/WHO maximum residue limits (WHO, 1984a).

WHO toxicity class: III
EC hazard: Xn, R22, R43, R50, R53, N
ADI (JMPR): 0.02 mg/kg

Environment and ecotoxicology

In soil, tecnazine is rapidly lost through evaporation (WHO, 1984a). The toxicity to aquatic organisms is as follows: the 96-h LC_{50} rainbow trout is 0.37 mg/L and *Daphnia magna* 48-h LC_{50} is 0.58 mg/L.

Chloroneb

Chemical identification

Class: chlorinated dimethoxybenzene
Structural formula: see Figure 6.3

Figure 6.3 Chloroneb (demosan)

Molecular weight: 207.1
Common name: chloroneb
IUPAC and CAS name: 1,4-dichloro-2,5-dimethoxybenzene
CAS no.: 2675-77-6

Uses and mechanism of activity

Chloroneb is a broad spectrum systemic fungicide taken up by the roots, and used on various fruit and vegetable crops as a wettable or dry application powder or dust. Mechanism of fungal toxicity may be related to inhibition of DNA polymerization (Phillips, 2001).

Toxicology

Acute toxicity

The rat oral LD_{50} is $>11\,000$ mg/kg and the rabbit percutaneous LD_{50} is >5000 mg/kg.

Primary irritation

Chloroneb is non-irritant to guinea pig skin.

Dicloran

Chemical identification

Class: chloronitroaniline
Structural formula: see Figure 6.4
Molecular weight: 207.0
Common name: dicloran
IUPAC name: 2,6-dichloro-4-nitroaniline
CAS name: 2,6-dichloro-4-nitrobenzene amine
Synonym: ditranil
CAS no.: 99-30-9

Figure 6.4 Dicloran

Uses and mechanism of activity

Dicloran is a protective fungicide producing hyphen distortion, which interferes with RNA transfer to ribosomes, and inhibits protein synthesis. It is used in the control of *Botrytis*, *Monilinia*, *Rhizopus*, *Sclerotinia*, and *Sclerotium* on fruits and vegetables, at application rates of 0.8–3.0 kg/ha and is available as dispersible powder, suspension concentrate, and wettable powder.

Toxicology

Acute toxicity

The rat oral LD_{50} is 4040–8000 mg/kg while that in the guinea pig LD_{50} is 1450 mg/kg. The mouse percutaneous LD_{50} is >5000 mg/kg and that in the rabbit >2000 mg/kg. The rat inhalation 1-h LC_{50} is >21.6 mg/L.

Chronic toxicology and oncogenicity

In a 2-year dietary study, 2000 ppm did not increase mortality but did increase final body weight. At 100 ppm there were no significant effects (Johnston, Woodard, and Cronin, 1968).

Developmental toxicology

Given in the diet to pregnant rabbits at 100 or 1000 ppm over gd 8–16, no teratogenic effects were noted (Edwards, Ferry, and Temple, 1991).

Reproductive toxicology

A three-generation study in rats receiving 100 ppm of dicloran in the diet showed no adverse reproductive effects (Johnston, Woodard, and Cronin, 1968).

Metabolism and toxicokinetics

After rats were dosed orally or by intraperitoneal injection at 20–40 mg/kg, 70–77 per cent was excreted in urine by 24 h, and 80–90 per cent by 48 h. Only about 1 per cent was excreted in faeces (Mate, Ryan, and Wright, 1967). Rats fed dicloran excrete a trace of unchanged material in urine, together with 3,5-dichloro-4-aminophenol and 2,6-dichloro-*p*-phenylenediamine. Using [^{14}C]dicloran, in the first 24 h 70 per cent activity was associated with the phenol, and 2.4 per cent with the diamine (Gallo, Bachmann, and Golberg, 1976; Mate, Ryan, and Wright, 1967).

Human and occupational toxicology

Dicloran is metabolized to dichloroaminophenol, an uncoupler of oxidative phosphorylation, which, may result in hyperpyrexia, liver injury, and corneal opacities. However, although corneal opacities have been demonstrated experimentally in dogs fed dichloran (Earl *et al.*, 1971), they have not been seen in occupationally exposed workers (Edwards, Ferry, and Temple, 1991).

WHO toxicity class: III
ADI (JMPR): 0.01 mg/kg

Environment and ecotoxicology

Principal plant metabolites are 4-amino-3,5-dichlorophenol, 4-amino-2,6-dichloroacetanilide, and 4-amino-2,6-dichoroaniline (Tomlin, 2000). In soil there is microbial degradation to 4-amino-2,6-dichloroaniline. Soil $DT_{50} = 39–78$ days (pH 6.3–7.1; organic matter 0.7–3.4 per cent). $K_{oc} = 760$ (sand, pH 6.0).

The oral LD_{50} for bobwhite quail is 900 mg/kg and the 5-day dietary LC_{50} is 1435 mg/kg. For mallard ducks the oral LD_{50} was >2000 mg/kg and the 5-day dietary LC_{50} 5960 mg/kg. For aquatic organisms 96-h LC_{50} are for rainbow trout 1.6 mg/L, for bluegill sunfish 37 mg/L, and for goldfish 32 mg/L. For *Daphnia* the 48-h LC_{50} is 2.07 mg/L. For bees, the contact LD_{50} is 0.18 mg/bee, while for worms (*Eisenia foetidia*) 1-day LD_{50} is 885 mg/kg.

Hexachlorobenzene

This has been withdrawn for human use, but included for consumer historical interest.

Chemical identification

Class: chlorinated benzene
Structural formula: see Figure 6.5
Molecular weight: 284.8
Common, IUPAC and CAS names: hexachlorobenzene (HCB)

Cl Cl Cl Cl Cl Cl

Figure 6.5 Hexachlorobenzene

Synonyms: perchlorobenzene; pentachlorophenol chloride
CAS no.: 118-74-1
EEC no.: 204-273-9

Uses and mechanism of activity

Hexachlorobenzene is a selective fungicide on fungal spores. It is used for the treatment of seeds for control of common bunt and dwarf bunt of wheat. Formulated as dry powder and water dispersible powder.

Toxicology

Acute toxicity

The rat oral LD_{50} is 3500–10 000 mg/kg, that in the mouse LD_{50} 4000 mg/kg, and in rabbit LD_{50} 2600 mg/kg.

Short-term and subchronic toxicology

Rats given dietary doses of 10 mg/kg for 15 weeks developed hyperparathyroidism, osteosclerosis, and renal tubular damage (Andrews *et al.*, 1989). The nephropathy is α_{2u}-dependant and specific to the male rat (Bouthillier, Greselin, and Brodeur, 1991).

Chronic toxicology and oncogenicity

Male rats fed diet containing 40 ppm HCB for 130 weeks developed chronic nephritis (Hathaway, Proctor, and Hughes, 1993). In chronic studies with mice, liver tumours were seen following dosing at 12–24 mg kg^{-1} day^{-1}, but not at 6 mg kg^{-1} day^{-1} (IARC, 1979). Hepatomas, hepatocellular carcinomas, bile duct adenomas, and renal cell adenomas were found in a rat chronic dietary study (IARC, 1987).

Genetic toxicology

HCB is not genotoxic. It did not induce dominant lethal mutations, chromosome aberrations were not seen *in vitro* in Chinese hamster ovary (CHO) cells, and was not mutagenic in an Ames test (IARC, 1987).

Developmental toxicology

HCB crosses the human and rodent placenta, and residues have been detected in human milk, adipose tissue, and umbilical cord blood (Hathaway, Proctor, and Hughes, 1993). Teratogenic effects were not seen in rats given up to 120 mg kg^{-1} day^{-1} over the period of organogenesis, but mice developed cleft palate and renal agenesis (IARC, 1979).

Reproductive toxicology

Fertilization and gestational indices were not adversely affected in rats receiving HCB up to 40 ppm (Arnold, 1985).

Human and occupational toxicology

An epidemic of about 3000 cases of porphyria cutea tarda resulted from the consumption of HCB-contaminated wheat in Turkey (Schmidt, 1960). The mortality rate was 10 per cent. Daily consumption of HCB was estimated at 50–200 mg/day. The majority of affected patients were children, with a high mortality for infants of mothers who ingested contaminated bread. Manifestations included abnormal porphyrin metabolism, hyperpigmentation, liver enlargement, hirsutes, short stature (in affected children), thyroid enlargement, painless arthritis, weakness, paraesthesia, cogwheel rigidity, and myotonia (Hathaway, Proctor, and Hughes, 1993). The syndrome of HCB poisoning was called 'black sore' because of characteristic skin blistering, infection with pigmented scars, and alopecia (Wray, Muftu, and Dugramace, 1962). The severe clinical findings and mortality lead to withdrawal of HCB.

WHO toxicity class: Ia
EC hazard rating: R45, T, R48/25, N, R50, R53
ACGIH assessment: $TWA_8 = 0.002\,mg/m^3$; skin and A3 notation
IARC assessment: 2B

Quintozene

Chemical identification

Class: chlorinated mononitrobenzene
Molecular weight: 295.36
Structural formula: see Figure 6.6
Common name: quintozene
IUPAC and CAS names: pentachloronitrobenzene
CAS no.: 82-68-8
EEC no.: 201-435-0

Figure 6.6 Quintozene

Uses and mechanism of activity

Quintozene is used as a seed and soil contact fungicide for a large variety of vegetable and field crops at an applicaton rate of 1–1.5 kg/ha. It is available as dispersible powder, emulsifiable concentrate, granular formulation, suspension concentrate, and wettable powder.

Toxicology

Acute toxicity

Estimates of the rat oral LD_{50} range from >5000 mg/kg to >30 000 mg/kg (WHO, 1984b), while the dog LD_{50} is >2500 mg/kg (Berkowitz *et al.*, 1976). The rat intraperitoneal LD_{50} is 5000 mg/kg (WHO, 1984b). The rabbit percutaneous LD_{50} is >5000 mg/kg and the rat 4-h inhalation: LC_{50} is >1.7 mg/L.

Primary irritation

Quintozene is not irritating to rabbit skin, but is slightly irritating to rabbit eye. An occupational case of keratoconjunctivitis, with resolution within a month, was reported (Fujita, Suzuki, and Ochiai, 1976).

Sensitizing potential

Allergic contact dermatitis has been reported (Edwards, Ferry, and Temple, 1991).

Short-term repeated and subchronic toxicology

Rats were fed quintozene over 3 months at daily dosages of 0, 63.5, 635, 1250, 2500, or 5000 mg/kg. Growth and survival were decreased in males and females at the high dosage and males at 2500 mg/kg. Liver enlargement occurred at all dosages, but histology showed only fine vacuolization of hepatocytes. With dogs fed diets containing 25, 200, or 1000 mg/kg quintozene for 1 year, there were no effects on survival, body weight, or haematology. Histopathological findings were limited to hepatocyte enlargement, but not dosage-related (Finnegan *et al.*, 1958). Rhesus monkeys fed quintozene at 2 mg/kg for 70 days had normal histology, haematology, and serum cholesterol (Muller *et al.*, 1978).

Chronic toxicology and oncogenicity

Dogs fed diets containing 500, 1000, or 5000 mg quintozene/kg for 2 years had dosage-related liver pathology. At 5000 mg/kg there was fibrosis, narrowing of liver cell cords, increased size of periportal areas, and leukocyte infiltration.

At 500 and 1000 mg/kg the effects were similar but less marked. Bone marrow atrophy with reduced haematopoiesis was seen at 5000 mg/kg (WHO, 1984b). Purebred beagle dogs received diets containing quintozene at 5–1080 mg/kg for 2 years without effects on food consumption, body weight, mortality, blood chemistry, urinalysis, or oestrus cycle. Haematocrit values were reduced at 18 months in dogs receiving 30 and 180, but not 1080 mg/kg. Liver weights were increased at the higher dosage. Histopathology revealed reversible hepatorenal effects at 2 years in the 180 and 1080 mg/kg groups (Borzelleca *et al.*, 1971). Osborne–Mendel rats and $B6C3F_1$ mice fed quintozene (97 per cent pure) in the diet for 78 weeks had no statistically significant increase in neoplasms (NCI, 1978b).

Genetic toxicology

Quintozene was not positive in an Ames test for metabolic activation (Mohn, 1971), or in reverse mutation assays with *Salmonella typhimurium* or *Escherichia coli* (Moriya *et al.*, 1983). There was no activity in a dominant lethal test in mice (Van Logten, 1977).

Developmental toxicology

Quintozene was not teratogenic in rats dosed up to 1563 mg/kg (Courtney, Copeland, and Robbins, 1976; Jordan and Borzelleca, 1973; Khera and Villeneuve, 1975). Although technical quintozene (87 per cent pure) given over gd 7–11 produced renal agenesis in C57BI/6 mice at 500 mg/kg, this was not seen with pure (99 per cent) material (Courtney, Copeland, and Robbins, 1976). HCB, a major contaminant, was implicated as the renal teratogen.

Reproductive toxicology

Rats were fed a diet containing 0, 5, 50, or 500 mg/kg until the F/3b litters were weaned. There were no effects on fertility, gestation, viability, or lactation (WHO, 1984b).

Metabolism and toxicokinetics

Major routes of excretion of ingested quintozene are as unchanged material in faeces and metabolites in urine. The amounts of faecal material suggest that absorption by the peroral routes is limited and species-dependent. With rabbits, 46–62 per cent of an oral dose was eliminated unchanged in faeces in 72 h (Betts, James, and Thorpe, 1955). In sheep, 80 per cent was eliminated unchanged in faeces (Avraham and White, 1976). However, in primates only 7.4 per cent of an oral dose was eliminated as faecal quintozene (Koegel *et al.*, 1979). Major urine metabolites were pentachloroaniline and mercapturic acids (WHO, 1984b). Several studies show little bioaccumulation (WHO, 1984b). For example, with Rhesus

monkeys given quintozene in diet for 2 mg/kg over 550 days, storage curves leveled out at 30–40 days with a plateau at 2–3 per cent of the dose (Muller *et al.*, 1978).

Human and occupational toxicology

The estimated human lethal dose is 500–5000 mg/kg. Although not a skin irritant, skin sensitization may develop (Edwards, Ferry, and Temple, 1991). With heavy over-exposure, methemoglobinaemia may occur (Phillips, 2001).

ADI (JMPR): 0.01 mg/kg
WHO toxicity class: III. WHO (1984b) do not regard the general population at risk from food residues, and note that occupational exposure has not caused serious adverse effects
EC hazard: Xi, R43
ACGIH assessment: $TWA_8 = 0.5\ mg/m^3$; A4 notation

Environment and ecotoxicology

In plants, quintozene is converted to pentachloroaniline, methylthiopentachlorobenzene, and various chlorophenylmethyl sulphoxides and sulphones (Tomlin, 2000). $T_{1/2}$ in soil is about 4.7–9.7 months (Wang and Broadbent, 1973). Soil biodegradation is to pentachloroaniline and methylthiopentachlorobenzene. K_{oc} values for sand: adsorption 2966, desorption 3285. A bioconcentration factor (BCF) of 238 was reported for *Pseudorasbora parva* using a flow-through system with quintozene concentrations ranging 5 to 20 g/L (Kanazawa, 1981). Avian toxicity values are for mallard ducks an LD_{50} of 2000 mg/kg and an 8-day dietary $LC_{50} > 5000$ ppm and for bobwhite quail an 8-day dietary $LC_{50} > 5000$ ppm. In aquatic organisms 96-h LC_{50} for rainbow trout were 0.55 ppm and for bluegill sunfish 0.1 ppm. For carp the 48-h LC_{50} was 10 mg/L. For *Daphnia* the 48-h LC_{50} was 0.77 mg/L and for shrimp and oyster, respectively, the 96-h LC_{50}s were 0.012 ppm and 0.029 ppm. Bees have a contact LD_{50} of $>100\ \mu g$/bee.

Pentachlorophenol

Pentachlorophenol (PCP) has been reviewed in detail by ATSDR (1994), Rao (1978), and WHO (1987).

Chemical identity

Class: chlorinated phenol
Structural formula: see Figure 6.7
Molecular weight: 266.3

OH
Cl Cl
Cl Cl
Cl

Figure 6.7 Pentachlorophenol

Common, IUPAC and CAS names: pentachlorophenol
CAS no.: 87-86-5
EEC no.: 201-778-6

Uses and mechanism of activity

PCP is used extensively to protect wood from fungal rot. PCP frequently has impurities including polychlorinated phenols, polychlorinated benzodioxins, and polychlorinated dibenzofurans. It is available as granule, oil-miscible liquid, and wettable powder.

Toxicology

Acute toxicity

Typical findings are (Ahlborg and Larrson, 1978; Borzelleca *et al.*, 1986; Gaines, 1969; Kehoe, Deichmann-Gruebler, and Kitzmiller, 1939; Renner, Hopper, and Gokel, 1986): Oral rat LD_{50} is 150 and 210 mg/kg, and specifically for male rats 146 mg/kg and for female rats 175 mg/kg. Oral LD_{50} values in mice LD_{50} are 129 mg/kg in males and 74 mg/kg in females. Signs include increased breathing rate, increased temperature, tremors, convulsions, loss of righting reflex, and asphyxial spasms. The intraperitoneal LD_{50} in mice is 59 mg/kg in males and 61 g/kg in females. The percutaneous rat LD_{50} is 320 mg/kg in males and 149 mg/kg in females.

Primary irritation

Corneal injury may result from splashes or vapour over-exposure (ATSDR, 1994).

Short-term and subchronic toxicology

Rats were given PCP in the diet at dosages of 25, 50, and 200 mg kg^{-1} day^{-1} for 12 weeks. At 50 and 200 mg kg^{-1} day^{-1} there was increased liver weight, haemoglobin concentration, haematocrit, and glucose; 25 mg kg^{-1} day^{-1} was a no

observed adverse effect level (NOAEL) (Knudsen *et al.*, 1974). In another rat study, diets containing technical or purified PCP were fed at 500 mg kg^{-1} day^{-1} for 8 months. The technical material slowed growth rate and produced hepatomegaly, porphyria, and increased aryl hydrocarbon hydroxylase, glucuronyl transferase, and cytochrome P450 activities. Pure material caused decreased growth rate and increased hepatic glucuronyl transferase activity (Goldstein *et al.*, 1977). In a further rat study, technical or purified PCP was given in the diet at 0, 20, 100, or 500 mg kg^{-1} day^{-1} for 8 months. The only effect with purified material was slight hepatomegaly at 500 mg kg^{-1} day^{-1}. However, technical material produced a dose-related increase in liver size with histopathological changes consisting of thickened centrilobular veins, pleomorphic hepatocytes, and cytoplasm vacuolation (Kimbrough and Linder, 1978). The results of these and other studies indicate repeated exposure toxicity to be due to impurities. Rats and rabbits were exposed by the respiratory route to PCP at concentrations of 2.97 and 28.9 mg/m^3 for 4 h/day for 4 months. At the highest concentration there was anaemia, leukocytosis, eosinophilia, and liver injury. At 2.97 mg/m^3 there were minimal decreases in cholinesterase and blood sugar (Demidenko, 1969).

Chronic toxicology and oncogenicity

In a rat 2-year dietary study using purified PCP, a NOAEL was established at 3 mg/kg. At 10 mg/kg there was pigment accumulation, and at 30 mg/kg there was decreased body weight gain and increased serum glutamate–pyruvate transaminase activity. There was no significant increase in tumour incidence (Schwetz *et al.*, 1978). Mice given PCP at the maximum tolerated dose (MTD) for 78 weeks did not show an increase in tumour incidence (Innes *et al.*, 1969). Male B6C3F$_1$ mice given diets containing 100 or 200 ppm technical grade PCP had increased adrenomedullary and hepatocellular neoplasms; female mice had increased incidences of haemangiosarcomas and hepatocellular neoplasms. Using another technical grade of material up to 600 ppm, males and females had increased incidences of adrenomedullary and hepatocellular neoplasms, and additionally females had hepatic and splenic haemangiosarcomas (NTP, 1989).

Genetic toxicology

Mutagenic activity did not occur in a *Salmonella typhimurium* assay without metabolic activation (Anderson, Leighty, and Takahashi, 1972), a sex-linked lethal test with *Drosophila melanogaster* (Vogel and Chandler, 1974), and a host-mediated assay (Schwetz *et al.*, 1978). A mammalian dominant lethal test was negative (Buselmaier, Rohrborn, and Propping, 1973). A mammalian spot test suggested a weak positive (not statistically significant), and with *Saccharomyces cerevisiae* there was an increase in mitotic gene conversion (Fahrig, Nilsson, and Rappe, 1978).

Developmental toxicology

Laboratory studies suggest that PCP is embryotoxic but not teratogenic, but the results may be complicated by impurities. Thus, whereas technical grade PCP dosed to female rats at 5 mg kg^{-1} day^{-1} over gd 6–15 caused no adverse effects, at 15 mg kg^{-1} day^{-1} there were fetal resorptions and delayed development, and at 35 mg kg^{-1} day^{-1} there was, additionally, maternal toxicity (Schwetz, Keeler, and Gehring, 1974). Delayed development was indicated by delayed skull ossification, supernumerary, fused, or missing vertebrae, and lumbar spurs. Purified PCP caused maternal toxicity and decreased fetal body weight at 30 mg/day, and delayed fetal development at 5 mg/kg. In a study in which female rats were given purified PCP 60 mg kg^{-1} day^{-1} on gd 8, 9 or 10, there were isolated instances of fetal dwarfism, exencephaly, macrophthalmia, and absence of tails. These effects were attributed to maternal hyperpyrexia (Larsen *et al*., 1975).

Reproductive toxicology

Technical grade PCP (95 per cent) was given in the diet to rats at 0, 5, 50, or 500 mg kg^{-1} day^{-1} from day 21, through breeding, gestation, and up to weaning 21 days post partum. There were significant decreases in survival to weaning at 5 mg kg^{-1} day^{-1}, but not at the higher doses (Exon and Keller, 1983). Purified PCP (30 mg kg^{-1} day^{-1}) was fed to male and female rats for 62 days premating, 15 days during mating, and to females during gestation and lactation. This resulted in decreased numbers of offspring, neonatal body weight, survival, and growth of weanlings. The NOAEL was 3 mg kg^{-1} day^{-1}. Male fertility was unaffected (Schwetz *et al*., 1978).

Metabolism and toxicokinetics

PCP is readily absorbed by the peroral, inhalation, and percutaneous routes, with highest concentrations occurring in the liver and kidney. Bioaccumulation is low. In rodents, metabolism is through oxidative conversion to tetrachlorohydroquinone, to a lesser extent to trichlorohydroquinone, and by glucuronic acid conjugation. Rats, mice, and primates excrete PCP and metabolites mainly in urine (rodents 62–83 per cent; primates 45–75 per cent) and to a lesser extent in faeces (rodents 4–34 per cent; primates 4–17 per cent) (WHO, 1987). Rats and mice eliminate PCP rapidly with a $t_{1/2}$ of 6–27 h. PCP binds to mitochondrial proteins and inhibits mitochondrial ATPase activity. It may have two independent effects on mitochondria, namely uncoupling oxidative phosphorylation and inhibiting ATPase (Stockdale and Selwyn, 1971). Thus, both the formation of ATP and the release of energy from the breakdown of ATP are prevented. An increase in cellular O_2 demand during the uncoupling of oxidative phosphorylation has been observed,

which gives rise to the initial increase in respiratory rate in some PCP poisoning cases (Mitsuda, Murakami, and Kawai, 1963; Wood *et al.*, 1983).

Human and occupational toxicology

PCP may be detected in adipose tissue, human milk, and blood. In one US survey, with blood and urine analyses from 6000 persons from 64 communities between 1976 and 1980, PCP was detected in the range 6–193 ppb with 79 urine samples. Occupationally exposed individuals may have blood concentrations ranging 83 to 57 600 ppb (ATSDR, 1994). Food and water supplies are a common environmental source of human PCP exposure (ATSDR, 1994), as is vapour inhalation by occupational exposure. PCP is an uncoupler of oxidative phosphorylation. It is effectively absorbed by the inhalation, percutaneous, and peroral routes of exposure. The $t_{1/2}$ for elimination from plasma is around 30 h, with 74 per cent eliminated as parent compound and 12 per cent as glucuronide conjugate (ATSDR, 1994). The minimum lethal dose of PCP by ingestion is estimated at 29 mg/kg (Ahlborg and Thunberg, 1980), although this depends on dosage, renal function, general health, and ambient temperature (Kozak *et al.*, 1979). Inhalation over-exposure to PCP may cause symptoms from irritation of the eyes, skin, throat, and respiratory tract. Hyperpyrexia, tachycardia, headache, ataxia, weakness, disorientation, diaphoresis, nausea, and vomiting may occur. Very high vapour exposures cause pulmonary oedema and death from cardiac arrest (Mason *et al.*, 1965; Robson *et al.*, 1969; Stevens and Richardson, 1979). Short-term massive over-exposure may also result in seizures, delirium, and coma (Phillips, 2001). Liver injury can occur. Corneal injury can result from splashes or vapour exposure. Short-term respiratory exposure may result in transient mild liver dysfunction (increased bilirubin, serum glutamate-pyruvate and oxaloacetic-pyruvate transaminases, and γ-glutamyl transferase) with abnormal porphyrin metabolism (increased uroporphyrin and δ-aminolevulinic acid) (Fielder *et al.*, 1982; Jirasek *et al.*, 1974; Kozak *et al.*, 1979). Reversible renal function changes (decreased creatinine clearance and phosphaturia) have been described (Begley *et al.*, 1977). There are no clinical indications of peripheral neuropathy, although exposed workers have been shown to have decreased sensory nerve conduction rates (Triebig *et al.*, 1981). Indications of immunological effects in exposed individuals include T-cell suppression and decreased IgA and IgE (McGovern, 1982; Ning, 1980). In this respect it is noted that mice fed diets containing 50 or 500 ppm technical grade PCP showed significant decreased immunocompetence as increased susceptibility to the growth of transplanted tumours (Kerkuliet, 1982). Autopsy features include alimentary tract inflammation with poisoning by ingestion, and pulmonary congestion, intra-alveolar haemorrhages, and pulmonary oedema following inhalation over-exposure. Spleno-, cardio- and hepato-megaly are frequently observed. Histopathology shows hepatic centrilobular fatty degeneration and necrosis, and renal tubular degenerative changes. There is

usually extreme rigor mortis (Bergner, Constantinidas, and Martin, 1965; Blair, 1961; Mason *et al.*, 1965; Robson *et al.*, 1969).

WHO toxicity class: Ib.
EC hazard rating: R40, T+, R26, T, R24/25, Xi, R36/37, N, R50, R53.
ACGIH assessment: $TWA_8 = 0.5\,mg/m^3$; skin, A3 notations; BEI, total urine PCP 2 mg/g creatinine, free plasma PCP 5 mg/L (ACGIH, 2003).
OSHA PEL = $0.5\,mg/m^3$, with skin notation.

Environment and ecotoxicology

The relatively high volatility and water solubility of PCP have led to widespread contamination of the environment. For example, in urban areas PCP concentrations in the range 5.7–7.8 ng/m^3 have been detected (WHO, 1987). The magnitude of absorption of PCP is pH-dependant, increasing with acidity (Green and Young, 1970; Kaufman, 1976). PCP is subject to photochemical degradation in water, organic solvents, and on solid surfaces. Photolytic products include lower chlorophenols, chlorinated dihydroxybenzenes, and non-aromatic fragments, with the rate of photodecomposition increasing with increasing pH (Wong and Crosby, 1978, 1981). The microbial degradation of PCP results in variable metabolites, depending on the bacterial strain, but include tetrachlorophenols, trichlorophenols, dichlorobenzene, and tetra/trichloroanisoles (Kuwatsuka and Igarashi, 1975; Rott, Nitz, and Klorte, 1979; WHO, 1987). Major metabolic processes include methylation, acylation, dechlorination, and hydroxylation (Kaufman, 1978; Reiner, Chu, and Kirsch, 1978; Rott, Nitz, and Klorte, 1979). Most aquatic species are affected by PCP concentrations <1 mg/L. In general, reproductive and juvenile stages are the most sensitive with LC_{50} values as low as 0.01 mg/L (WHO, 1987). Low oxygen, low pH, and increased temperature increase toxicity. Fresh water fish show BCFs up to 1000 compared with <100 in marine fish.

Toxicity values for aquatic organisms include the following (Adema and Vink, 1981; Conklin and Rao, 1978; Dijik, 1977; Gupta and Rao, 1982; WHO, 1987).

Rainbow trout	48-h $LC_{50} = 0.17$ mg/L
Brown trout	48-h $LC_{50} = 0.17$ mg/L
Chlorella pyrenoldosa	96-h $EC_{50} = 7$ mg/L (growth)
Chlorella ovalis	96-h $EC_{50} = 5.5$ mg/L (growth)
Lymnaea acuminata	96-h $LC_{50} = 0.16$ mg/L
Crangon crango	96-h $LC_{50} = 0.11$ mg/L
Palaemonetes pugio	96-h $LC_{50} = 0.44$ mg/L
Pimephales promelas	96-h $LC_{50} = 0.6$ mg/L
Cyprinodon varioatus	96-h $LC_{50} = 0.223$ mg/L
Lagodon rhomboides	96-h $LC_{50} = 0.053$ mg/L
Cyprinus carpio	96-h $LC_{50} = 0.01$ mg/L
Lebistes reticulatus	96-h $LC_{50} = 0.97$ mg/L

Dinocap

Chemical identity

Class: dinitrophenol derivative
Structural formula: see Figure 6.8
Molecular weight: 364.3
Common name: dinocap
IUPAC name: 2,6-dinitro-4-octylphenyl crotonates and 2,4-dinitro-6-octylphenyl crotonates (octyl is mixture of 1-methylheptyl, 1-ethylhexyl and 1-propylphenyl)
CAS name: (*E*)-2-(1-methylheptyl)-4,6-dinitrophenyl 2-buteonate
Synonym: 2,4-dinitro-6-(1-methyl-*n*-heptyl)phenyl croteonate
CAS no.: 131-72-6 (single compound); 39300-45-3 (mixed isomers)
EEC no.: 254-408-0 (mixed isomers)

Uses and mechanism of activity

Dinocap is a contact fungicide having curative and protective actions, used for control of powdery mildews on fruit and vegetables. It is available as dispersible powder, emulsifiable concentrate, and wettable powder. The commercial material is now known to be a mixture of 1.0 part 4-octyl isomers to 2.0–2.5 parts of 6-octyl isomers.

Toxicology

Acute toxicity

In rats the oral LD_{50} in males is 980 mg/kg and females 1190 mg/kg, in male rabbits 2000 mg/kg, and in the dog $LD_{50} = 100$ mg/kg. The percutaneous rabbit LD_{50} is 2350 mg/kg, while the rat 4-h inhalation $LC_{50} = 0.36$ mg/kg. The intravenous LD_{50} in mice is 2.3 mg/kg (Larson *et al.*, 1959).

Figure 6.8 Dinocap

Primary irritation

Dinocap is a moderate eye irritant.

Sensitizing potential

A repeated insult skin patch test in 50 human volunteer subjects showed a skin sensitizing potential (Larson *et al*., 1959).

Chronic toxicology and oncogenicity

Dinocap had no tumourigenic potential when fed to two strains of mice (Innes *et al*., 1969).

Developmental toxicology

Pregnant CD-1 mice were dosed 0, 6, 12, and 25 $mg\,kg^{-1}\,day^{-1}$ over gd 7–16. At 25 $mg\,kg^{-1}\,day^{-1}$ there was post-natal mortality, and 24 per cent of the survivors showed torticollis. Of the 12 mg/kg group, 6 per cent had torticollis. In pregnant rats dosed up to 100 mg/kg the only effect was reduced maternal weight gain (Gray *et al*., 1986). Pregnant mice were dosed with dinocap (0–120 $mg\,kg^{-1}\,day^{-1}$) over gd 7–16. At 120 mg/kg there was complete fetal mortality, and at 80 mg/kg live fetuses per litter were reduced and resorptions increased. Dosage-related cleft palate was found in fetuses at 5 (0.4 per cent), 20 (23.6 per cent), 40 (75.5 per cent), and 80 (74.1 per cent) $mg\,kg^{-1}\,day^{-1}$ (Rogers *et al*., 1986).

Human and occupational toxicology

Dinocap is an uncoupler of oxidative phosphorylation. It is irritating to skin and causes sensitization (Larson *et al*., 1959; Mazella di Bosco, 1970). The EPA requires protective equipment including protective suits, goggles, face shields, and in some cases enclosed dispersing vehicles.

ADI (JMPR): 0.008 mg/kg
WHO toxicity class: III
EC hazard: Xn, R22, Xi, R38

Environment and ecotoxicology

Principal soil metabolite is 2,4-dinitro-6-(2-octyl)phenol. $DT_{50} = 4.5–6.1$ days by microbial degeneration.

Dichlorophen

Chemical identification

Class: chlorinated biphenyl
Structural formula: see Figure 6.9
Molecular weight: 269.1
Common name: dichlorophen
IUPAC name: 4,4′-dichloro-2,2′-methylenediphenol
CAS name: 2,2′-methylene[4-chlorophenol]
Synonyms: antiphen; 2,2′-dihydroxy-5,5′-dichlorodiphenylmethane
CAS no.: 97-23-4
EEC no.: 202-567-1

Uses and mechanism of activity

Dichlorophen is a contact fungicide for control of moss, red thread, *Fusarium*, and dollar spot and is available as emulsifiable concentrate and soluble concentrate.

Toxicology

Acute toxicity

The oral LD_{50} for rats is 2690 mg/kg, for mice 1000 mg/kg, for guinea pigs 1250 mg/kg, and for dogs 2000 mg/kg.

Human and occupational toxicology

Dichlorophen is an uncoupler of oxidative phosphorylation; 2.7 μM dichlorophen produced a 50 per cent increase in mitochondrial ATPase activity (Nakaue, Caldwell, and Buhler, 1972). Allergic contact dermatitis has been reported (Reynolds *et al*., 1989).

WHO toxicity class: III
EC hazard rating: Xn, R22, Xi, R36, N, R50, R53

Figure 6.9 Dichlorophen

Dithiocarbamate fungicides

The carbamates pesticides are N-substituted esters of carbamic acid, having the general formula $R^1NH—CO—OR^2$, where R^1 and R^2 are aliphatic or aromatic moieties. Dithiocarbamates are the disulphur analogues of carbamates, characterized by the presence of the grouping shown in Figure 6.10.

Depending on the monoamine used in synthesis, mono- or di-alkyldithiocarbamates are formed. Reaction of carbon disulphide with diamines gives two terminal dithiocarbamate groups linked by an alkylene bridge. Based on these considerations, the dithiocarbamate fungicides are divisible into three major groups, namely monoalkyldithiocarbamates (methyl), dialkydithiocarbamates (methyl and ethyl), and ethylenebisdithiocarbamates. Their general characteristics have been reviewed in WHO (1988). Both alkyl and ethylene dithiocarbamates form salts with metals. The fungicides in the metallodithiocarbamate group derive their names from the attached metal. In general they are of low environmental persistence, low to moderate acute mammalian toxicity, and low cumulative potential. A principal effect of dithiocarbamates is inhibition of SH-containing enzymes (e.g. α-ketoglutaroxidase, pyruvate dehydrogenase, succinic dehydrogenase). Typical clinical presentations for acute over-exposure are headache, weakness, excitability, nausea, vomiting, diarrhoea, abdominal discomfort, dizziness, and coma (Kaloyanova and Batawi, 1991). Carbon disulphide (and its metabolites) are the only compounds common to the metabolism of dithiocarbamate fungicides. Poisoning by various dithiocarbamates and that produced by carbon disulphide are similar (Kane, 1970; Rainey and Neal, 1975).

Dithiocarbamates

This group of fungicides, which are carbamic acid derivatives, includes metam, ferbam, thiram, and ziram. One feature is their ability to inhibit alcohol dehydrogenase, leading to potentiation of toxicity by co-exposure to ethanol. They are not, or are poor, cholinesterase inhibitors. Most have a potential to cause thyroid gland dysfunction, with decreased uptake of ^{131}I and plasma T_4 and increased TSH (Edwards, Ferry, and Temple, 1991; Marrs, 1999). Using a bioassay based on the inhibition of growth of *Escherichia coli*, Segovia *et al.* (2002) found a statistically significant regression between the degree of toxicity, expressed as EC_{50}, and different parameters related to the molecular structure of dithiocarbamates (including the molecular weight of the

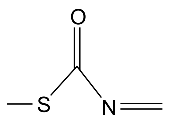


Figure 6.10 Characteristic grouping in dithiocarbamates

carbamate, the molecular weight of the smallest radical, and the number of benzene rings).

Metam-sodium

Chemical identification

Class: monoalkyldithiocarbamate
Structural formula: see Figure 6.11
Molecular weight: 129.2
IUPAC and EEC name for metam: methyldithiocarbamic acid
CAS no.: for metam 144-54-7, for metam-sodium 137-42-8
EEC no.: for metam 205-632-2, for metam-sodium 205-293-0

Uses and mechanism of activity

Metam-sodium acts, in part, by decomposition to methyl *iso*cyanate. Metam-sodium is a soil fumigant.

Toxicology (metam-sodium)

Acute toxicity

Oral LD_{50}s are in male rats 1800 mg/kg, female rats 1700 mg/kg, and mice 285 mg/kg. In rabbits the percutaneous LD_{50} is 1300 mg/kg, while in rats the 4-h inhalation LC_{50} is >4.7 mg/L.

Primary irritation

Metam-sodium is corrosive to the skin.

Human and occupational toxicology

WHO toxicity class: II
EC hazard rating: Xn, R22, R31, C, R34, R43, N, R50, R53

Na'
S
S−
NH

Figure 6.11 Metam-sodium

Environment and ecotoxicology

Metam-sodium rapidly decomposes in soil to methyl isocyanate; $DT_{50} = 23$ min–4 days. The oral LD_{50} in bobwhite quail is 500 mg/kg. In mallard ducks and Japanese quail the 5-day dietary LC_{50} is >5000 mg/kg. In aquatic organisms the 96-h LC_{50} for guppy is 4.2 mg/L, for bluegill sunfish 0.39 mg/L, and for rainbow trout 35.2 mg/L. For *Daphnia* the 48-h EC_{50} is 2.3 mg/L.

Ferbam

Chemical identification

Class: dialkylmetallodithiocarbamate
Structural formula: see Figure 6.12
Molecular weight: 416.5
Common name: ferbam
IUPAC name: iron *tris*(dimethyldithiocarbamate); iron(III)dimethyldithiocarbamate; ferric dimethyldithiocarbamate
CAS name: *tris*(dimethylcarbamodithioato-S,S′)iron
CAS no.: 14484-64-1
EEC no.: 238-484-2

Uses and mechanism of activity

Ferbam is a foliar fungicide with protective action and is used in the control of scab on various fruits and blue mould on tobacco. Ferbam is available as wettable granules.

N S S N Fe'2 S⁻ ⁻S S S⁻ N

Figure 6.12 Ferbam

Toxicology

Acute toxicity

The oral rat LD_{50} is >4000 mg/kg and the rabbit percutaneous LD_{50} is >4000 mg/kg. By inhalation, the rat 4-h LC_{50} is >0.4 mg/L.

Primary irritation

Slightly irritating to rabbit eye.

Short-term repeated and subchronic toxicology

Rats given a diet containing 0.5 per cent ferbam for 30 days resulted in 18/20 dying. There was no significant gross or microscopic pathology (Hodge, 1952).

Human and occupational toxicology

ADI (JMPR): 0.003 mg/kg
WHO toxicity class: III
EC hazard rating: Xi, R36/37/38, N, R50, R53
OSHA PEL: 15 mg/m^3 (total dust)
ACGIH assessment: TWA_8 10 mg/m^3; skin notation (ACGIH, 2003)

Environment and ecotoxicology

Principal plant metabolite is the dimethylamine salt of dimethyldithiocarbamic acid. Soil DT_{50} (aerobic) = 4.9 days. Avian toxicity values are as follows: Japanese quail LD_{50} is >2000 mg/kg, bobwhite quail: dietary LC_{50} is 2940 ppm, mallard duck dietary LC_{50} is >3640 ppm. Toxicity values for aquatic organisms are a 96-h LC_{50} of 10.02 mg/L for zebra fish and a 48-h LC_{50} of 0.09 mg/L for *Daphnia*. For worms the 14-day LC_{50} is 625 mg/kg soil.

Thiram

Chemical identity

Class: dialkyldithiocarbamate
Structural formula: see Figure 6.13
Molecular weight: 240.4
Common name: thiram
IUPAC name: tetramethylthiuram disulphide; *bis*(dimethylthiocarbamoyl) disulphide
CAS name: tetramethyl thioperoxydicarbonic diamide
Synonyms: tetramethylthiuram disulphide; thiuram

Figure 6.13 Thiram

CAS no.: 137-26-8
EEC no.: 205-286-2

Uses and mechanism of activity

Thiram is a general use contact fungicide with protective action. It is used to control *Botrytis* on fruit and vegetables and in seed treatment. It is available as dispersible powder, flowable and suspension concentrates, water dispersible granules and powder, and liquid seed treatment.

Toxicology

Acute toxicity

Rat oral LD_{50} is 2600–4000 mg/kg, that in the mouse is $LD_{50} = 4$ g/kg, and that in the rabbit is $LD_{50} = 210$ mg/kg. Signs of toxicity include ataxia, hypoactivity, laboured breathing, clonic convulsions, and death within 2–7 days. The percutaneous rabbit LD_{50} is >2000 mg/kg. The rat 4-h inhalation LC_{50} is 4.42 mg/L.

Primary irritation

Thiram causes slight skin erythema. It is moderately irritant to rabbit eye.

Sensitizing potential

Sensitization has been demonstrated in the guinea pig (Brusilovskiy and Fialkovskiy, 1973). Human cases of allergic contact dermatitis have been described (Dalvi, 1988; Fogh and Puck-Steen, 1992).

Chronic toxicology and oncogenesis

Thiram was not carcinogenic in rats receiving thiram chronically by gavage or in the diet (IARC, 1991). However, it has been noted that thiram can react with nitrite

under mildly acidic conditions to form *N*-nitrosodimethylamine. Administration of 500 ppm thiram with 700 ppm sodium nitrite in the diet for 2 years led to a high incidence of nasal tumours in comparison with either controls or with animals receiving the materials alone (Lijinsky, 1984). In a dietary study, at 2500 ppm all rats died within 17 weeks. Mortality was not increased at 1000 ppm but there was weakness, ataxia, and hind limb paralysis. A few animals developed these effects with 300 ppm, and 100 ppm was a NOAEL (Lehman, 1965). In an 80-week study, hind limb paralysis and atrophy was seen in female rats receiving 67 mg kg^{-1} day^{-1}; a marginal effects dosage (slight growth depression and fatty infiltration of the pancreas) was 5 mg kg^{-1} day^{-1} (Lee, Russell, and Minor, 1978). The hind limb ataxia and paralysis due to thiram dosing is associated with chromatolysis and pyknosis of the ventral horn neurones of the lower lumbar cord region, and with demyelinating changes in the sciatic nerve (Lee and Peters, 1976).

Developmental toxicology

Thiram was teratogenic (skeletal malformations) at acutely maternally toxic doses (250 mg/kg) in hamsters. Also, it produced skeletal malformations in mice given maternally toxic dosages over gd 6–17 (Robens, 1969; Roll, 1971). The incidence and severity of malformations caused by thiram was reduced by the co-administration of L-cysteine at 10 mg kg^{-1} day^{-1} (Matthiaschk, 1973).

Reproductive toxicology

Given in the diet for 13 weeks at 132 mg/kg, thiram caused reduced fertility (IARC, 1991). Given by gavage at 25 mg kg^{-1} day^{-1} for 90 days there was a significant increase in testicular weight with minimal testicular histological changes (Mishra, Srivastava, and Raizada, 1993). In mice given an acute subcutaneous dose (1000 mg/kg) or 5 repeated doses of 250 mg/kg there was a significant increase in the frequency of abnormal spermatozoa (Hemavathi and Rahiman, 1993).

Metabolism and toxicokinetics

Carbon disulphide production occurs in a dose-related manner, and is reduced by the administration of the inhibitor SKF 525A. Carbon disulphide may contribute to the hepatotoxicity of thiram (Dalvi and Deoras, 1986). A dosage of 0.05 LD_{50}/day for 6 days or 0.02 LD_{50} for 4 months caused an increase in liver weight and hexabarbital sleeping time (Kolycheva, Nadzhimutdinoz, and Muzrabekov, 1973; Nadzhimutdinoz, Kamilov, and Muzrabekov, 1974). Dosing rats with thiram at 60 mg/kg caused a decrease in cytochrome P450 and benzphentamine *N*-demethylase activities, with increased serum sorbitol dehydrogenase and serum glutamate-oxaloacetic transaminase (Dalvi and Deoras, 1986).

Human and occupational toxicology

Thiram may cause a low incidence of allergic contact dermatitis (Dalvi, 1988; Phillips, 2001; Tomlin, 2000; Wilson, 1969). A skin reaction (erythema, pruritis and urticaria) without other systemic effects may occur in chronically exposed workers after alcohol ingestion (Shelley, 1964). Ingestion may cause focal necrosis of the alimentary tract (Phillips, 2001). Following absorption, thiram causes nausea, dizziness, headache, confusion, ataxia, abdominal pain, vomiting, diarrhoea, and flaccid paralysis (Dalvi, 1988). These effects may be accentuated by concurrent ingestion of ethanol. Intolerance to alcohol has been observed in workers exposed to thiram, who develop facial flushing, tachycardia, hypotension, and dizziness. These effects may be related to inhibition of alcohol dehydrogenase by thiram, and the accumulation of acetaldehyde. Reports of goiters in workers handling thiram-treated seeds have appeared in the Russian literature; workplace thiram concentrations ranged from 0.2 to 5.0 mg/m^3 (D'yachuk, 1972) or from 0.03 to 5.2 mg/m^3 (Kaskevich and Bezuglyg, 1973).

ADI (JMPR): 0.01 mg/kg
WHO toxicity class: III
EC hazard rating: R40, Xn, R20/22, Xi, R36/37, R43
ACGIH assessment: TWA_8 1 mg/m^3, A4 notation (ACGIH, 2003)

Environment and ecotoxicology

The major plant metabolite is the dimethylamine salt of dimethyldithiocarbamic acid. Soil $DT_{50} = 0.5$ days (sandy, pH 6.7). Toxicity values for birds include:

Male ring-necked pheasants	oral $LD_{50} = 673$ mg/kg
Mallard ducks	oral $LD_{50} > 2800$ mg/kg
Redwing blackbirds	oral $LD_{50} > 100$ mg/kg
Ring-necked pheasants	8-day $LC_{50} > 5000$ mg/L
Mallard ducks	8-day $LC_{50} > 5000$ mg/L
Bobwhite quail	8-day $LC_{50} > 3950$ mg/L
Japanese quail	8-day $LC_{50} > 5000$ mg/L

Toxicity values for aquatic organisms include:

Bluegill sunfish	96-h $LC_{50} = 0.0445$ mg/L
Rainbow trout	96-h $LC_{50} = 0.128$ mg/L
Daphnia	48-h $LC_{50} = 0.21$ mg/L

For bees, the peroral LD_{50} is >2000 μg/bee and the contact LD_{50} is 73.7 μg/bee. For worms, the 14-day LC_{50} is 540 mg/kg soil.

Ziram

Chemical identification

Class: dialkylmetallodithiocarbamate
Structural formula: see Figure 6.14
Molecular weight: 305.0
Common name: ziram
IUPAC name: zinc *bis*(dimethyldithiocarbamate)
CAS name: (T-4)-*bis*(dimethyldithiocarbamato-S,S′)zinc
CAS no.: 137-30-4
EEC no.: 205-288-3

Uses and mechanism of activity

Ziram is a contact foliar fungicide with protective function, which is available as wettable granules, powder, and liquid. It is used to control fungi in various fruits and vegetables.

Toxicology

Acute toxicity

Oral rat LD_{50} is 2068 mg/kg in males and 1400 mg/kg in females. The rabbit LD_{50} is 400 mg/kg and that in the guinea pig is 100–150 mg/kg. The percutaneous rabbit LD_{50} is >2000 mg/kg, while the rat 4-h inhalation LC_{50} is 0.07 mg/L. The intraperitoneal LD_{50}s are rat 23 mg/kg (male) and 33 mg/kg (female), and mouse 17 mg/kg.

Primary irritation

Ziram is minimally irritating to rabbit skin and highly irritating to rabbit eye.

Chronic toxicology and oncogenicity

In a rat 2-year dietary study, at 2500 ppm, but not 250 ppm, growth was retarded. Minimal thyroid and testicular changes were seen at the highest concentration, but tumour incidence was not increased (Hodge *et al.*, 1956).

Figure 6.14 Ziram

Human and occupational toxicology

Low vapour pressure excludes exposure to vapour. Occupational inhalation exposure is to airborne dust, which results in irritation of the eyes, throat, and respiratory tract. Ingestion can cause focal necrosis of the alimentary tract.

ADI (JMPR): 0.003 mg/kg
WHO toxicity class: III
EC hazard rating: Xn, R22, Xi, R36/37/38
IARC classification: group 3

Environment and ecotoxicology

The major plant metabolite is dimethylamine salt of dimethyldithiocarbamic acid. Soil $DT_{50} = 42$ h (aerobic). For bobwhite quail, the $LD_{50} = 97$ mg/kg. For aquatic organisms, toxicity values include, for rainbow trout, a 96-h LC_{50} of 1.9 mg/L and for *Daphnia* a 48-h EC_{50} of 0.048 mg/L. For bees, the LD_{50} is >100 μg/bee. For worms, the 7-day LC_{50} is 190 mg/kg soil.

Ethylene bisdithiocarbamates

This group includes maneb, mancozeb, nabam, trimanzone, and zineb. Major occupational health problems have been with irritancy and, with maneb and zineb, allergic contact dermatitis. Unlike the dithiocarbamates, they do not inhibit alcohol dehydrogenase. A major concern has been that ethylene thiourea (ETU) may be a contaminant or decomposition product. The carcinogenic activity of ETU has resulted in significant regulatory activity and concern being given to this generic group of fungicides. Discussions have been concerned with both ETU as a possible metabolite and/or the formation of nitrosamines (Edwards, Ferry, and Temple, 1991).

Maneb

Chemical identification

Class: metallo-alkylenebisdithiocarbamate
Structural formula: see Figure 6.15
Molecular weight: 265.3
Common name: maneb
IUPAC name: manganese ethylenebis(dithiocarbamate)
CAS name: [1,2-ethanediylbis[carbamodithioato](2-)]manganese
CAS no.: 12427-38-2
EEC no.: 235-654-8

Mn'2

S⁻ ⁻S

S= =S

NH NH

Figure 6.15 Maneb

Uses and mechanism of activity

Maneb is a fungicide with protective action against a wide range of fungi in fruit and vegetables, by foliar application, or seed treatment. Primary uses are control of late blight on potatoes, tomatoes, fruit, vegetables, and ornamental plants. Available as suspension concentrate, water dispersible granules, wettable powder or seed treatment.

Toxicology

Acute toxicity

The rat oral LD_{50} is >5000–6750 mg/kg, the percutaneous rat and rabbit LD_{50} is >5000 mg/kg, and the rat inhalation 4-h LC_{50} is >3.8 mg/L.

Acute intraperitoneal injection of maneb to rats (20–200 mg/kg) reduced the cold-induced endogenous TSH response. There was no effect on TSH secretion after TRH or on serum T_3 or T_4. It was considered that maneb inhibits rat TSH secretion at the hypothalamic level by inhibition of dopamine β-hydroxylase (Laisi *et al.*, 1985).

Primary irritation

Maneb is a mild skin irritant and moderate eye irritant in rabbits.

Sensitizing potential

Maneb is a skin sensitizer by the guinea pig maximization procedure, with cross-reactivity to zineb (Matsushita, Arimatsu, and Nomura, 1976).

Short-term and subchronic toxicology

Dogs fed maneb at 200 mg kg^{-1} day^{-1} developed flaccid paraplegia and were moribund within 3–7 months. After 1 year at 75 mg kg^{-1} day^{-1} there was persistent weight loss and anorexia. Signs included tremors, weakness, depression of coordination and reflexes, and hindlimb hypotonia. There was also evidence of

impaired renal function at 75 and 200 mg kg^{-1} day^{-1}. No detectable effects occurred at 2 mg kg^{-1} day^{-1} (Edwards, Ferry, and Temple, 1991).

Chronic toxicology and oncogenicity

Maneb was not tumorigenic in two strains of mice at the MTD (Innes *et al.*, 1969). Another mouse study showed a significant increase in lung adenomas (Balin, 1970).

Developmental toxicology

Maneb was not teratogenic in mice, but rat studies have shown a teratogenic potential, possibly related to zinc deficiency (Edwards, Ferry, and Temple, 1991; Larsson *et al.*, 1976).

Metabolism and toxicokinetics

Given to rats by gavage at 333–390 mg/kg, 55 per cent of the dose was excreted as metabolites in faeces and urine within 5 days. Excreta contained ethylenediamine, ethylene-bisthiuram monosulphide, and ethylenebisthiourea (Seidler *et al.*, 1970).

Human and occupational toxicology

Occupational allergic contact dermatitis has been described (Nater, Terpstra, and Bleumink, 1979; O'Malley, 1997).

ADI (JMPR): 0.03 mg/kg
WHO toxicity class: III
EC hazard rating: Xi, R37, R43

Environment and ecotoxicology

The principal metabolite in plants is ETU, which rapidly undergoes further metabolism. Maneb is rapidly degraded in the environment by hydrolysis, oxidation, photolysis, and metabolism. The soil DT_{50} is about 25 days. The 8-day dietary LC_{50} for mallard ducks is >10 000 mg/kg diet, and for bobwhite quail >10 000 mg/kg diet. The 48-h LC_{50} for carp is 1.8 mg/L.

Mancozeb

Chemical identity

Class: metallo-alkylenebisdithiocarbamate
Structural formula: see Figure 6.16
Common name: mancozeb
IUPAC name: manganese ethylenebis(dithiocarbamate) complex with zinc salt

Figure 6.16 Mancozeb

CAS name: [[1,2-ethanediylbis[carbamodithioato]](2-)]manganese with [[1,2-ethanediylbis[carbamodithioato]](2-)]zinc
Synonym: manzeb
CAS no.: 8018-67-6
ISO definition: complex of zinc and maneb containing 20% of manganese and 2.55% of zinc, the salt present being stated

Uses and mechanism of activity

Mancozeb is used to control a large variety of fungal diseases (including blight, leaf spot, rust downy mildew, scab) in field crops, fruits, nuts, vegetables, and ornamentals. It is used by foliar application or seed treatment, and is available as dispersible powder, powder for seed treatment, suspension concentrate, water dispersible granules, and wettable granules.

Toxicology

Acute toxicity

The oral rat LD_{50} is >5000 mg/kg. The percutaneous LD_{50} is >10 000 mg/kg in rat and >5000 mg/kg in rabbit. The rat 4-h inhalation LC_{50} is >5.14 mg/L.

Primary irritation

Mancozeb is a mild skin irritant and moderate eye irritant in the rabbit.

Human and occupational toxicology

ADI (JMPR): 0.03 mg/kg
WHO toxicity class: III
EC hazard assessment: Xi, R37, R43

Environment and ecotoxicology

Mancozeb is metabolized extensively in plants to ETU, ethylenethiuram monosulphide, ethylenethiuram disulphide, and sulphur. Rapid environmental degradation is by hydrolysis, oxidation, and photolysis. Soil DT_{50} is 6–15 days. $K_{oc} > 2000$.

Avian toxicity values include: 10-day dietary LC_{50} for mallard ducks is >6400 mg/kg and Japanese quail is >3200 mg/kg. In fish 48-h LC_{50}s are 9.0 mg/L in goldfish, 2.2 mg/L in rainbow trout, 5.2 mg/L in catfish, and 4.0 mg/L in carp. For bees, the LC_{50} is 0.193 mg/bee.

Zineb

Chemical identification

Class: metallo-alkylenebisdithiocarbamate
Structural formula: see Figure 6.17
Molecular weight: 275.8
Common name: zineb
IUPAC name: zinc ethylenebis(dithiocarbamate)
CAS name: [[1,2-ethanediylbis[carbamodithioato]](2-)]zinc
CAS no.: 12122-67-7
EEC no.: 235-180-1

Uses and mechanism of activity

Zineb is a folial fungicide with protective actions. It is used in the control of downy mildews on vines, hops, and various fruits and vegetable. It is available as dispersible and wettable powders.

Figure 6.17 Zineb

Toxicology

Acute toxicity

Various estimates of the rat oral LD_{50} are 4400, >5000, and 6100 mg/kg. The percutaneous rat LD_{50} is >2500 mg/kg.

Primary irritation

Zineb is a mild skin irritant and slight eye irritant to rabbits.

Sensitizing potential

The guinea pig maximization procedure indicated a strong sensitizing potential for zineb and cross reactivity with maneb (Matsushita, Arimatsu, and Nomura, 1976).

Chronic toxicology and oncogenicity

In a rat 2-year dietary study 10 000 ppm caused increased mortality in females. Renal pathology and thyroid gland hyperplasia were seen (Edwards, Ferry, and Temple, 1991). In dogs, a dietary level of 10 000 ppm caused an increase in thyroid gland weight with histological evidence of hyperplasia. At 2000 ppm there were no detectable effects on the thyroid gland (Smith *et al*., 1953). In two strains of mice, zineb, at the maximum tolerated dose, was not tumorigenic (Innes *et al*., 1969).

Human and occupational toxicology

Sulphhaemoglobinaemia and acute haemolytic anaemia with Heinz bodies was described in patients with glucose-6-phosphate dehydrogenase deficiency following zineb exposure (Pinkhas *et al*., 1963). Allergic contact dermatitis has been reported (O'Malley, 1997).

ADI (JMPR): 0.03 mg/kg
EC hazard rating: Xi, R37, R43

Environment and ecotoxicology

A major plant metabolite is ETU. In aquatic organisms, the LC_{50} in perch is 2 mg/L and in roach is 6–8 mg/L.

Benzimidazole/thiabendazole fungicides

This class is confused since the individual classes are closely related. Sometimes, however, the benzimidazoles are classified separately but in an overlapping manner, creating confusion. They are nitrogen heterocyclic compounds, with parent structures of thiabendazole and/or benzimidazole. Included in this overall group are benomyl, thiabendazole, thiophanate, thiophanate–methyl, mebendazole, carbedazim, imazalil, and fuberidazole. Benomyl, carbendazim, thiophanate, and thiophanate–methyl are sometimes referred to (and classified) as benzimidazole carbamates. Many of these fungicides inhibit mitochondrial fumarate reductase, reduce glucose transport, and uncouple oxidative phosphorylation. Inhibition of microtubule polymerization by binding to α-tubulin is a primary action, and specific high affinity binding to host α-tubulin occurs at significantly lower concentrations than does mammalian protein binding (Phillips, 2001).

Benomyl

Chemical identification

Class: benzimidazole carbamates
Structural formula: see Figure 6.18
Molecular weight: 290.3
Common name: benomyl
IUPAC name: methyl 1-(butylcarbamoyl)benzimidazol-2-yl carbamate
CAS name: methyl[1-[(butylamino)carbonyl]-1*H*-benzimidazol-2-yl] carbamate
Synonym: methyl-1-(butylcarbamoyl)-2-benzimidazole-carbamate
CAS no.: 17804-35-2
EEC no.: 241-775-7

Uses and mechanism of activity

Benomyl is a systemic fungicide with curative and protective actions and is absorbed through leaves and roots with acropetal translocation. Benomyl is effective

Figure 6.18 Benomyl

against *Ascomycetes*, *Fungi imperfecti*, and some *Basidiomycetes* in cereals, fruit, rice, and vegetables and is formulated as wettable powder.

Toxicology

Acute toxicity

The rat oral LD_{50} is >5000 mg/kg, the percutaneous rabbit LD_{50} is >5000 mg/kg, and the 4-h inhalation rat LC_{50} is >2 mg/L.

Primary irritation

Benomyl is mildly irritant to rabbit skin and eye.

Sensitizing potential

A guinea pig maximization procedure showed skin sensitizing potential (Matsushita and Aoyama, 1979). Allergic contact dermatitis has been recorded in greenhouse workers (Savitt, 1972) and confirmed by Matsushita and Aoyama (1981).

Short-term repeated and subchronic toxicology

In a 90-day nose-only inhalation study, rats were exposed to 0, 10, 50, or 200 mg/m^3 for 5 h/day for 5 days/week. At 45 days, and the end of the exposure period, there was degeneration of the olfactory epithelium in all males and females at 200 mg/m^3, and a few males of the 50 mg/m^3 group (Warheit *et al.*, 1989). It is relevant that benomyl may produce an impairment of ciliary function, with decreased beat frequency (Kucera *et al.*, 1995). For rats fed diets over 32 days containing 0, 5000, 10 000, or 15 000 ppm, the only dose-related toxicity was a decrease in body weight gain and food consumption (Von Burg, 1993).

Chronic toxicology and oncogenicity

No toxicity occurred in rats given diets containing up to 150 $mg\,kg^{-1}\,day^{-1}$ (Phillips, 2001). In mice given benomyl for 2 years in the diet (0, 500, 1500, 5000, and 7500 ppm) males had liver tumours at the lowest two dosages but not the higher dosages. Benign liver tumours were seen in all groups of female mice (Von Burg, 1993).

Developmental toxicology

The fetuses of rats given 62.5 mg/kg by gavage over gd 6–15 had craniofacial abnormalities (Ellis *et al.*, 1988).

Reproductive toxicology

In a rat three-generation reproduction study, there were no effects on reproduction or lactation at 150 mg kg^{-1} day^{-1}. In another rat three-generation study, the highest level tested (2500 ppm) produced no effects on reproduction or lactation, and no pathology in weanling pups of the F_{3b} generation (Sherman, Culik, and Jackson, 1975). Male rats give benomyl by gavage at 0, 1, 5, 15, or 45 mg kg^{-1} day^{-1} showed decreased testicular weight and sperm production at 45 mg kg^{-1} day^{-1} at 76–79 days (Linder *et al.*, 1987).

Metabolism and toxicokinetics

Benomyl is metabolized to carbendazim followed by slow conversion to 2-aminobenzimidazole. Hydroxylation also occurs, with the principal metabolite (5-hydroxybenzimidazole carbamate) being converted to *O*- and *N*-conjugates. Benomyl and metabolites are excreted in urine and faeces within a few days, with no tissue accumulation (Tomlin, 2000).

Human and occupational toxicology

Benomyl is unlikely to result in any significant acute health problem, but may cause skin sensitization (O'Malley, 1997; Savitt, 1972; Van Joost, Neafs, and van Ketel, 1983; van Ketel, 1976).

ADI (JMPR): 0.1 mg/kg
WHO toxicity class: III
EC hazard rating: R40
ACGIH assessment: $TWA_8 = 10\ mg/m^3$; A4 notation (ACGIH, 2003)

Environment and ecotoxicology

In plants, metabolism occurs to the relatively stable carbendazim followed by slow degradation to 2-aminobenzimidazole. Benomyl is rapidly converted to carbendazim in the environment. $DT_{50} = 2$ h (water), 19 h (soil). $K_{oc} = 1900$. The 8-day dietary LC_{50} is >10 000 mg/kg for mallard ducks and bobwhite quail. For aquatic species 96-h LC_{50} for rainbow trout is 0.27 mg/L, for goldfish is 4.2 mg/L, and for guppy 3.4 mg/L. For *Daphnia* the 48-h LC_{50} is 640 g/L. For bees, the contact LD_{50} is >50 μg/bee, and for worms the 14-day $LC_{50} = 10.5$ mg/kg.

Thiabendazole

Chemical identification

Class: benzimidazole
Structural formula: see Figure 6.19

Figure 6.19 Thiabendazole

Molecular weight: 201.3
Common name: thiabendazole
IUPAC name: 2-(thiazol-4-yl)benzimidazole; 2-(1,3-thiazol-4-yl)benzimidazole
CAS name: 2-(4-thaizolyl)-1*H*-benzimidazole
Synonym: (4-[2-benzimidazolyl]thiazide)
CAS no.: 149-79-8
EEC no.: 205-725-8

Uses and mechanism

Thiabendazole is a systemic fungicide with protective and curative actions. It binds to tubulin and inhibits mitosis, and hence impairs fungal growth and development. Thiabendazole can be used as a post-harvest fungicide, and seed treatment and foliar application to fruits and vegetables can be carried out. It is available as suspension concentrate, soluble concentrate, and wettable powder.

Toxicology

Acute toxicity

The rat oral LD_{50} is 3100 mg/kg, the mouse LD_{50} is 3600 mg/kg, and the rabbit oral LD_{50} is 5800 mg/kg. The percutaneous rabbit LD_{50} is >2000 mg/kg and the rat inhalation LC_{50} is >0.5 mg/L.

Primary irritation

Thiabendazole is not irritant to rabbit skin or eye.

Short-term and subchronic toxicology

Dogs given 20, 100, or 200 mg kg^{-1} day^{-1} orally survived for 2 years, with few signs and normal weight gain. At 200 and 100 mg kg^{-1} day^{-1} dogs developed a normocytic normochromic anaemia with recovery by the end of the study (Edwards, Ferry, and Temple, 1991).

Metabolism and toxicokinetics

Perorally, thiabendazole is rapidly absorbed and distributed, with 90 per cent elimination within 24 h (65 per cent urine, 25 per cent faeces) (Tomlin, 2000).

Human and occupational toxicology

Ingestion may cause nausea, abdominal discomfort, vomiting, diarrhoea, dizziness, drowsiness, headache, vertigo, hyperexcitability, convulsions, and hepatorenal toxicity (Phillips, 2001).

ADI (JMPR): 0.1 mg/kg
WHO toxicity class: III

Environment and ecotoxicology

Soil DT_{50} = 33 days (20°C, 0.1 mg/kg soil), 120 days (20°C, 1 mg/kg soil). Aquatic photolysis DT_{50} = 29 h (pH 5). The oral LD_{50} = 19 mg/kg in bobwhite quail and 5-day dietary LC_{50}s in bobwhite quail and mallard ducks are >5620 mg/kg. In aquatic species, 96-h LC_{50}s are 19 mg/L in bluegill sunfish, 0.55 mg/L in rainbow trout, and 0.34 mg/L in shrimp. In *Daphnia* the 48-h EC_{50} is 0.81 mg/L and in *Selenastrum* the 96-h EC_{50} is 9 mg/L; the NOAEL is 3.2 mg/L. For worms, the LC_{50} is >500 mg/kg (soil).

Thiophanate–methyl

Chemical identification

Class: benzimidazole
Structural formula: see Figure 6.20
Molecular weight: 342.4
Common name: thiophanate−methyl
IUPAC name: dimethyl-4,4′-(*o*-phenylene)bis(3-thioallophanate)
CAS name: dimethyl[1,2-phenylene bis(iminocarbonothioyl)]bis(carbamate)
CAS no.: 23564-05-8
EEC no.: 245-740-7

Uses and mechanism

Thiophanate−methyl is a systemic fungicide with protective and curative actions. It is absorbed by leaves and roots and is effective against a wide range of fungal

S O HN N H O H N H N O S O

Figure 6.20 Thiophanate−methyl

pathogens. It is available as dispersible powder, paste, suspension concentrate, and wettable powder.

Toxicology

Acute toxicity

Oral LD_{50}s are: in rats 7500 mg/kg (males) and 6640 mg/kg (females), male mice 3510 mg/kg, male rabbits 2270 mg/kg, male guinea pigs 3640 mg/kg, and female guinea pigs 6700 mg/kg. The rat ip LD_{50} is 1640 mg/kg in males and 1140 mg/kg female, while the percutaneous rat LD_{50} is >10 000 mg/kg. The rat 4-h inhalation $LC_{50} = 1.7$ mg/L.

Primary irritation

Thiophanate–methyl is mildly irritant to rabbit skin and eye.

Short-term and subchronic toxicology

Mice fed a diet containing thiophanate–methyl for 6 months had a slight reduction in growth at 8000 ppm, with slight liver enlargement. The NOAEL was 1600 ppm. Similar results were obtained with rats (Noguchi, 1972).

Chronic toxicology and oncogenicity

In a 2-year rat dietary study, at 640 ppm there was a slight reduction in growth in both sexes, a slight increase in relative kidney weight in males, and slight enlargement in thyroid epithelial cells. 160 ppm was a NOAEL (Edwards, Ferry, and Temple, 1991).

Genetic toxicology

In vitro and *in vivo* cytogenetics studies, and a dominant lethal test, were negative (Makita, Hashimoto, and Noguchi, 1973; Noguchi, 1972).

Developmental toxicology

Mice received peroral dosages of thiophanate–methyl at 40, 200, 500, or 1000 mg/kg over gd 1–15. At 1000 mg kg^{-1} day^{-1} there was a slight reduction in the number of live fetuses per litter (9.7 versus 10.9 for controls), but no indications of a teratogenic effect. Lower dosages showed no abnormalities (Makita, Hashimoto, and Noguchi, 1973).

Reproductive toxicology

In a dietary three-generation reproduction study, the only effect was a slight reduction in growth at 640 ppm. The NOAEL was 160 ppm (Edwards, Ferry, and Temple, 1991).

Metabolism and toxicokinetics

In the rat, 61 per cent of ingested thiophanate–methyl is excreted in urine and 35 per cent in faeces within 90 min. Thiophanate–methyl is metabolized by cyclization to carbendazim. The principal metabolite in the rat is 5-hydroxybenzimidazol-2-carbamate (Tomlin, 2000).

Human and occupational toxicology

ADI (JMPR): 0.98 mg/kg
WHO toxicity class: III
EC hazard rating: R40, N, R50, R53

Environment and ecotoxicology

In plants, cyclization occurs to form carbendazim and this also occurs in soil and water under the influence of UV radiation. Carbendazim then degrades to 2-aminobenzimidazole and 5-hydroxy-2-aminobenzimidazole. Soil absorption $K_d = 1.2$. The oral LD_{50} is >5000 mg/kg for Japanese quail. For aquatic species, 48-h LC_{50}s are 7.8 mg/L for rainbow trout, 11.0 mg/L for carp, 0.8 mg/L for *Chlorella*, and 20.2 mg/L for *Daphnia*. Bees have a topical LD_{50} of >100 μg/bee.

Carbendazim

Chemical identification

Class: benzimidazole
Structural formula: see Figure 6.21
Molecular weight: 191.2

Figure 6.21 Carbendazim

Common name: carbendazim; carbendazime; carbendazol
IUPAC name: methyl benzimidazol-2-ylcarbamate
CAS name: methyl 1*H*-benzimidazol-2-ylcarbamate
Synonym: MBC
CAS no.: 10605
EEC no.: 234-232-0

Uses and mechanism

Carbendazim inhibits β-tubulin synthesis. It is a systemic fungicide with curative and protective functions and is absorbed through roots with acropetal translocation. Carbendazim is used to control various fungi in cereals, oilseed rape, and sugar beet. It is available as oil dispersible powder, suspension concentrate, wettable granules and powder, and solution concentrate.

Toxicology

Acute toxicity

The oral rat LD_{50} is $>15\,000$ mg/kg and that in the dog >2500 mg/kg. The intraperitoneal LD_{50} in male rats is 7320 mg/kg. The percutaneous rat LD_{50} is >2000 mg/kg and rabbit LD_{50} is $>10\,000$ mg/kg.

Primary irritation

Carbendazim is not irritant to rabbit skin or eyes.

Human and occupational toxicology

ADI (JMPR): 0.03 mg/kg
WHO toxicity class: III
EC hazard rating: R40

Environment and ecotoxicology

In soil, 2-aminobenzimidazole is a minor metabolite. Soil $DT_{50} = 8–23$ days. Avian toxicity figures include an oral LD_{50} of 5826–15 595 mg/kg for quail. In aquatic species 96-h LC_{50}s include carp 0.61 mg/L, rainbow trout 0.83 mg/L, bluegill sunfish 17.25 mg/L, and guppy >8 mg/L. In *Daphnia* the 48-h LC_{50} is 0.13–0.22 mg/L. In *Scenedesmus subspicatus*, 72-h EC_{50} is 419 mg/L and in *Selenastrum capricornutam* 1.3 mg/L. Bees have a contact LD_{50} of >50 µg/bee. The worm (*Eisenia foetidia*) 4-week LC_{50} is 6 mg/kg (soil).

Imazalil

Chemical identification

Class: imidazole
Structural formula: see Figure 6.22
Molecular weight: 297.2
Common name: imazalil; chloramizol; enilconazole (INN name)
IUPAC name: (±)-1-(β-allyloxy-2,4-dichlorophenylethyl)imidazole (±)-allyl 1-(2,4-dichlorophenyl)-2-imidazol-1-ylethyl ether
CAS name: (±)-1-[2-(2,4-dichlororphenyl)-2-(propenyloxy)ethyl]-1*H*-imidazole
CAS no.: 35554-44-0
EEC no.: 252-615-0

Uses and mechanism

Imazalil is an ergosterol biosynthesis inhibitor and a systemic fungicide with protective and curative actions. It is used to control a wide range of fungal diseases on fruit, vegetables, and ornamentals and is available as emulsifiable concentrate, water soluble powder, and soluble liquid.

Toxicology

Acute toxicity

The oral LD_{50} in Wistar rats is 343 mg/kg (males) and 227 mg/kg (females), in dogs it is >640 mg/kg. Signs include ataxia, piloerection, hypotonia, and tremors. The percutaneous rat LD_{50} is 4200–4880 mg/kg, while the rat 4-h inhalation LC_{50} is 16 mg/L.

Primary irritation

A dose of 640 mg/kg produced slight erythema, lasting for 1 week, in the rabbit (Edwards, Ferry, and Temple, 1991). In the rabbit eye slight transient irritation was observed.

Figure 6.22 Imazalil

Chronic toxicology and oncogenicity

In a chronic (24-month) study, Wistar rats were given imazalil in the diet up to 800 ppm. There were no effects on mortality, body weight, signs, haematology, or clinical chemistry. At necropsy there was a slight increase in some organ weights at the high dose, without significant histopathology (Edwards, Ferry, and Temple, 1991).

Genetic toxicology

Imazalil was not mutagenic in an Ames test, and did not produce dominant lethal effects up to 160 mg/kg (Edwards, Ferry, and Temple, 1991).

Developmental toxicology

Teratogenic effects were not seen in fetuses of Wistar rats fed imazalil in the diet at 5, 20, and 80 mg kg^{-1} day^{-1} over gd 6–15. Also a study in New Zealand White rabbits given imazalil by gavage at 0.63 and 2.5 mg kg^{-1} day^{-1} over gd 6–18 did not elicit teratogenic effects (Edwards, Ferry, and Temple, 1991).

Reproductive toxicology

Pregnant Wistar rats were given imazalil in the diet from day 16 of gestation and continued through the 3-week lactation period. At the highest dose there was 25 per cent mortality. In a three-generation reproduction study with 50, 200, or 800 ppm in the diet, no adverse effects occurred (Thienpont *et al.*, 1981).

Human and occupational toxicology

Occupational contact dermatitis has been described (van Hecke and De Vos, 1983).

ADI (JMPR): 0.03 mg/kg
WHO toxicity class: II
EC hazard rating: Xn, R20/22, X, R41, N, R50, R53

Environment and ecotoxicology

In plants, imazalil is converted to α-(2,4-dichlorophenyl)-1*H*-imidazole-1-ethanol. Soil $DT_{50} = 4–5$ days. Avian toxicity values include an oral LD_{50} of 2000 mg/kg in the ring-necked pheasant and an 8-day $LC_{50} > 2510$ mg/kg in mallard ducks. For aquatic species, 96-h LC_{50}s of 1.5 mg/L in rainbow trout and 4.04 mg/L in bluegill sunfish were observed. In *Daphnia* the 48-h LC_{50} is 3.5 mg/L. For bees, the oral LD_{50} is 40 μg/bee.

Fuberidazole

Chemical identification

Class: benzimidazole
Structural formula: see Figure 6.23
Molecular weight: 184.2
Common name: fuberidazole
IUPAC name: 2-(2-furyl)benimidazole
CAS name: 2-(2-furanyl)-1*H*-benzimidazole
Synonyms: furidazol; furidazole
CAS no.: 3878-19-1
EEC no.: 223-404-0

Uses and mechanism

Fuberidazole is a systemic fungicide with a specific action against *Fusarium*. It is a mitosis inhibitor and is available as a dry seed powder, a flowable concentrate, a solution for seed treatment, and a water dispersible powder.

Toxicology

Acute toxicity

The oral LD_{50} in the rat is 336 mg/kg and in the mouse is 650 mg/kg. The percutaneous rat LD_{50} is >5000 mg/kg. The rat 4-h inhalation LC_{50} (aerosol) is >0.3 mg/L.

Primary irritation

Fuberidazole is not irritating to rabbit skin or eyes.

Human and occupational toxicology

ADI: 0.006 mg/kg
WHO toxicity class: II
EC hazard rating: Xn, R22, N, R50, R53

Figure 6.23 Fuberidazole

Environment and ecotoxicology

Degradation in soils is rapid: $DT_{50} = 0.6–11$ days, and $DT_{90} = 5–64$ days. Avian toxicity values include an acute oral LD_{50} for Japanese quail of 500 mg/kg. For aquatic species, the 96-h LC_{50} for golden orfe is 16.2 mg/L and for rainbow trout 2.5 mg/L. For *Daphnia* the 48-h LC_{50} is 5.6 mg/L and for *Scenedesmus subspicatus* the EC_{50} is 0.49 mg/L. For worms (*Eisenia foetidia*) the LC_{50} is >1000 mg/kg (soil).

Chloroalkylthiodicarboximides

This group is sometimes referred to as R—SCCl compounds, and some classify them as a subdivision of nitrogen heterocyclics. However, nitrogen attached to the —$SCCl_3$ group is not required for fungitoxicity, and many S—$SCCl_3$, C—$SCCl_3$, or O—$SCCl_3$ compounds are fungicides. The group contains captan, captafol, and folpet, which generally are of low mammalian toxicity. However, because of their structural similarity to thalidomide, there has been concern about potential developmental toxicity. The overall negative findings for teratogenicity with chloroalkyldithiocarboximides has led to the conclusion that another region of the thalidomide molecule is responsible for the developmental toxicity potential. Thus, the glutaramide portion of the thalidomide molecule, which is not present in the fungicides, is possibly the teratogenic region, or it is a function of the configuration of the entire molecule (Edwards, Ferry, and Temple, 1991).

Captan

Chemical identification

Class: thiodicarboximide
Structural formula: see Figure 6.24
Molecular weight: 300.6
Common name: captan
IUPAC name: *N*-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide
CAS name: 3a,4,7,7a-tetrahydro2-[(trichloromethyl)thio]-1*H*-isoindole-1,3(2*H*)-dione
Synonym: *N*-trichloromethylmercapto-tetrahydrophthalimide

Figure 6.24 Captan

CAS no.: 133-06-2
EEC no.: 205-087-0

Uses and mechanism

Captan is a protective and curative fungicide and controls a wide range of fungal diseases in fruit. Captan is used as a seed treatment or root dip for *Pythium*, *Phoma*, and *Rhizoctonia* on maize, ornamentals, and vegetables. A major use is as a post-harvesting application to apples. It is available as dispersible powder, dry seed powder, water dispersible granules, wettable powder, and seed treatment.

Toxicology

Acute toxicity

Estimates of the oral LD_{50} in the rat are 9000, 12 600, and >17 000 mg/kg and in the mouse: 7480 mg/kg (male) and 7000 mg/kg (female) (Stevens, Farmer, and Dipasquate, 1978; Urbanek-Karloweka, 1975; Vashakdize, Mandzhyaladze, and Zhorjoliani, 1973). The intraperitoneal mouse LD_{50} is 142 mg/kg (male) and 462 mg/kg (female) (Stevens, Farmer, and Dipasquate, 1978). The percutaneous rabbit LD_{50} is >6500 mg/kg.

Primary irritation

Captan is a mild skin irritant in the rabbit and is corrosive to the rabbit eye.

Sensitizing potential

A 5 per cent sensitization rate was found by human skin patch testing, and a 10 per cent rate in human volunteers with a maximization test. Females were apparently more sensitive (Jordan and King, 1977).

Short-term repeated and subchronic toxicology

10 000 ppm included in the diet of rats caused depression of growth (Edwards, Ferry, and Temple, 1991). With rats given captan by gavage, the 100-day LD_{50} was 916 mg/kg. Signs, particularly in the first 3 weeks, included weight loss, decreased food consumption, increased water consumption, hypothermia, and prostration (Boyd and Carsky, 1971).

Chronic toxicity and oncogenicity

Captan was not oncogenic in two strains of mice receiving the MTD (Innes *et al.*, 1969). No significant increase in neoplasms was noted in rats at dietary levels of 2225 and 6050 ppm for 80 weeks (NCI, 1977).

Genetic toxicology

Captan is genotoxic *in vitro* but not *in vivo* (Legator and Zimmering, 1975; Moriya, Kato, and Shirasu, 1978); this aspect was reviewed in detail by Edwards, Ferry, and Temple (1991).

Developmental toxicity

Captan was not embryofetotoxic by gavage to New Zealand White rabbits at $80\,mg\,kg^{-1}\,day^{-1}$ over gd 7–12 (Fabro, Smith, and Williams, 1965). Negative teratogenicity studies in rats, hamsters, rabbits, and dogs have also reported by Kennedy, Fanther, and Calandra (1968) and Kennedy, Arnold, and Keplinger (1975).

Metabolism and toxicokinetics

Following peroral ^{14}C-captan, recovery was 51.8 per cent in urine, 22.8 per cent in expired air, 15.9 per cent in faeces, and 0.6 per cent in tissues. Urine metabolites included thiazolidine-2-thione-4-carboxylic acid, a salt of dithio(methanesulphonic acid), and the disulphide monoxide derivative of dithiobis(methanesulphonic acid). It was concluded that the gastrointestinal tract plays a major role in metabolism (DeBaum *et al*., 1974).

Human and occupational toxicology

Occupational allergic contact dermatitis has been described (Fregert, 1968; Hirano and Yoshikawa, 1982).

ADI (JMPR): 0.1 mg/kg
WHO toxicity class: III
EC hazard rating: R40, Xi, R36, R43
ACGIH assessment: $TWA_8 = 5\,mg/m^3$; sensitization and A3 notation (Notice of Intended Changes, ACGIH, 2003)

Environment and ecotoxicology

Captan is not phytotoxic. Soil $K_d = 3$–8 days (pH 4.5–7.2); $DT_{50} = 1$ day (pH 7.2, 25°C). Acute oral LD_{50}s of >5000 mg/kg were observed in mallard ducks and pheasants and of 2000–4000 mg/kg in bobwhite quail. In bluegill sunfish the 96-h LC_{50} is 0.072 mg/L, in harlequin fish 0.3 mg/L and in brook trout 0.034 mg/L. In *Daphnia* the 48-h LC_{50} is 7–10 ppm. Bees have a peroral LD_{50} of 91 μg/bee, and contact LD_{50} of 780 μg/bee.

Captafol

Chemical identification

Class: dithiocarboximide
Structural formula: see Figure 6.25
Molecular weight: 349.1
Common name: captafol; difolatan
IUPAC name: *N*-(1,1,2,2-tetrachloroethylthio)cyclohex-4-ene-1,2-dicarboximide; 3a,4,7,7a-tetrahydro-*N*-(1,1,2,2-tetrachloroethanesulphenyl)phthalimide
CAS name: 3a,4,7,7a-tetrahydro-2-[(1,1,2,2-tetrachloroethyl)thio]-1*H*-isoindole-1,3 (2*H*)-dione
Synonym: *cis-n*-[1,1,2,2-(tetrachloroethyl)thio]-4-cyclohexe-1,2-dicarboximide
CAS no.: 2425-06-1
EEC no.: 219-363-3

Uses and mechanism

Captafol is a non-systemic fungicide with protective and curative actions. It is a non-specific thiol reactant, inhibiting germination of spores and is a broad spectrum fungicide for fruit and vegetables, and seed protectant. Captafol is available as dispersible seed treatment powder, suspension concentrate, and wettable powder.

Toxicology

Acute toxicity

The rat oral LD_{50} is 6500 mg/kg and the rabbit percutaneous LD_{50} is >15 400 mg/kg. By inhalation the rat LC_{50} is >0.72 mg/L (males) and >0.87 mg/L (females).

Primary irritation

Captafol is a mild irritant to rabbit skin and corrosive to the rabbit eye.

Figure 6.25 Captafol

Sensitizing potential

Allergic contact dermatitis has been described (Stoke, 1979) in which photodermatitis is possible.

Chronic toxicology and oncogenicity

In a rat chronic study, 1500 and 5000 ppm caused increased mortality, decreased growth rate, and enlarged livers. Histology showed liver cell vacuolization with mononuclear cell infiltration, and renal tubules showed giant cells with large irregular nuclei. At 500 ppm (males and females) and 250 ppm (males) there was liver enlargement at 12 months. However, at the end of 2 years there was no liver enlargement, and no increase in tumour incidence (Edwards, Ferry, and Temple, 1991). In a dog chronic study there was vomiting and diarrhoea during the first 4 weeks at 100 and 300 mg kg^{-1} day^{-1}, with slight anaemia and growth retardation at 2 years. At 30 mg kg^{-1} day^{-1} there was increase in liver and kidney weights, but haematology and liver function tests were normal. A NOAEL was established at 10 mg kg^{-1} day^{-1} (Edwards, Ferry, and Temple, 1991).

Genetic toxicology

In a dominant lethal study in which male mice were given 1.5 or 3.0 mg/kg captafol by intraperitoneal injection there was no increase in early embryonic death. Similarly, rats dosed orally at 125 or 250 mg kg^{-1} day^{-1} for 14 days did not show a dominant lethal effect (Kennedy, Arnold, and Keplinger, 1975).

Developmental toxicology

No teratogenic effects were seen in the fetuses of rhesus monkeys fed diets containing captafol at 6.25–25 mg kg^{-1} day^{-1} from gd 22 to 32 (IARC, 1976). Also, no teratogenic effects were seen in rabbits dosed at 37.5–150 mg kg^{-1} day^{-1} over gd 6–16, or rats dosed at 100 or 500 mg kg^{-1} day^{-1} over gd 6–15 (Kennedy, Fanther, and Calandra, 1968).

Reproductive toxicology

In a rat three-generation study there was a slight reduction in pup weight at weaning at 1000 ppm, with statistical significance for the F_{1b}, F_{3a}, and F_{3b} animals, but not for F_{2a} and F_{2b}. Otherwise, there were no abnormalities (Kennedy, Fanther, and Calandra, 1968).

Human and occupational toxicology

Captafol can cause skin sensitization. Slight to marked erythematous dermatitis with eyelid oedema has been described following the occupational handling of

captafol (Edwards, Ferry, and Temple, 1991; Verhagen, 1974). Exposure to dust may result in respiratory tract irritation.

WHO toxicity class: Ia
EC hazard rating: R45, R43, N, R50, R53
ACGIH assessment: $TWA_8 = 0.1\ mg/m^3$, with skin and A4 notation (ACGIH, 2001)

Environment and ecotoxicology

In plants captafol is hydrolysed to dichloroacetic acid and tetrahydrophthalimide, with the latter being degraded to tetrahydrophthalimic acid, phthalic acid, and ammonia. In pheasants the 10-day dietary LC_{50} is $> 23\,070$ mk/kg (diet) and in mallard ducks $> 101\,700$ mg/kg (diet). For rainbow trout the 96-h LC_{50} is 0.5 mg/L, for goldfish 3.0 mg/L and for bluegill sunfish 0.15 mg/L. For *Daphnia*, the 96-h LC_{50} is 3.34 ppm.

Folpet

Chemical identification

Class: dithiocarboximide
Structural formula: see Figure 6.26
Molecular weight: 296.6
Common name: folpet
IUPAC name: *N*-(trichloromethylthio)phthalimide *N*-(trichloromethanesulphenyl) phthalimide
CAS name: 2-[(trichloromethyl)thio]-1*H*-isoindole-1,3(2*H*)-dione
Synonym: phaltan
CAS no.: 133-07-3
EEC no.: 205-088-6

Uses and mechanism

Folpet is a foliar fungicide with protective action. It is a non-specific thiol reactant used for the control of downy mildews, powdery mildews, leaf spot diseases, scab,

O
N—S
Cl
O Cl Cl

Figure 6.26 Folpet

excoriosis, black rot, white rot, *Gloeosporium* rots, *Botrytis*, *Alterneria*, *Pythium*, and *Rhizoctonia* in pome fruit, stone fruit, soft fruit, citrus fruit, hops, various vegetables, and ornamentals. Folpet is available as dispersible powder, suspension concentrate, water dispersible granules, and powder.

Toxicology

Acute toxicity

Estimates of the rat oral LD_{50} are >9000 mg/kg to 11 g/kg, while the percutaneous rabbit LD_{50} is >4500 mg/kg. The rat 4-h inhalation LC_{50} is 1.89 mg/L.

Primary irritation

Folpet is a mild to moderate skin irritant, and moderate eye irritant in the rabbit.

Metabolism and toxicokinetics

In vitro, folpet has a $t_{1/2}$ of about 1 min in human blood, with rapid degradation to phthalimide and phthalamic acid (EPA, 1987).

Human and occupational toxicology

ADI (JMPR): 0.1 mg/kg
WHO toxicity class: III
EC hazard rating: R40, Xi, R36, R43

Environment and ecotoxicology

Major metabolites in plants are phthalimide, phthalic acid, and phthalamic acid. $DT_{50} = 4.3$ days (soil) and 0.7 h (water). Folpet is strongly adsorbed to soil, $K_{oc} = 304\text{–}1164$. For mallard ducks, the acute oral LD_{50} is >2000 mg/kg. For bees, the oral LD_{50} is >236 μg/bee, and contact LD_{50} is >200 μg/bee.

Azoles

Most fungicides in this class are triazoles, being 5-membered rings containing three N atoms in the ring. They are systemic fungicides. In general they inhibit ergosterol biosynthesis (sterol demethylase inhibitors) leading to disruption of cell wall synthesis. Some classification systems refer to this group as ergosterol

biosynthesis-inhibiting fungicides (EBIFs). They induce cytochrome P450 which may enhance the oxidation of some organophosphates (OPs). In respiratory exposure studies, liver hypertrophy and enzyme induction have been demonstrated. Hyperactivity secondary to EBIF exposure, particularly with triadimefon and triadimenol, is due to effects on CNS catecholamines, notably dopamine (Walker and Mailman, 1993). Some classify imazalil with this group.

Cyproconazole

Chemical identification

Class: triazole
Structural formula: see Figure 6.27
Molecular weight: 291.8
Common name: cyproconazole
IUPAC name: (2*RS*,3*RS*;2*RS*,3*SR*)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol
CAS name: α-(4-chlorophenyl)-α-(1-cyclopropylethyl)-1*H*-1,2,4-triazol-1-ethanol
CAS no.: 94361-06-5

Uses and mechanism

Cyproconazole is a sterol demethylase inhibitor and a systemic fungicide with protective, curative, and eradicant actions. It is absorbed rapidly with acropetal translocation. It is used as a foliar systemic fungicide for control of *Septoria*, rust, powdery mildew, *Rhynchosporium*, *Cercospora*, and *Ramularia* in cereals and sugar beet (at 60–100 g/ha). It is also used for rust, *Mycena*, *Sclerotinia*, and *Rhizoctonia* in coffee and turf. It is available as a suspension and soluble concentrate, and water dispersible granules.

N N N OH Cl

Figure 6.27 Cyproconazole

Toxicology

Acute toxicity

Oral LD_{50}s are 1020 mg/kg for male rats, 1333 mg/kg for female rats, 200 mg for male mice, and 218 mg/kg for female mice. The percutaneous rabbit LD_{50} is >2000 mg/kg. The rat 4-h inhalation is $LC_{50} > 5.65$ mg/L.

Primary irritation

Cyproconazole is not irritating to rabbit skin, and is a minor irritant to rabbit eye.

Human and occupational toxicology

WHO toxicity class: II
EC hazard rating: R22

Environment and ecotoxicology

The major plant residue is cyproconazole. There is moderately rapid soil degradation; DT_{50} is about 3 months. Avian acute oral LD_{50} for Japanese quail is 150 mg/kg. Eight-day dietary LC_{50}s are 816 mg/kg (diet) for Japanese quail and 1197 mg/kg (diet) for mallard duck. Aquatic organism 96-h LC_{50} toxicity values include: 18.9 mg/L for carp, 19 mg/L for trout, and 21 mg/L for bluegill sunfish. In *Daphnia* the 48-h LC_{50} is 26 mg/L. For bees, the contact LD_{50} is >0.1 mg/bee and the peroral LD_{50} is >1 mg/bee.

Diniconazole

Chemical identification

Class: triazole
Structural formula: see Figure 6.28
Molecular weight: 326.2

Figure 6.28 Diniconazole

Common name: diniconazole
IUPAC name: (*E*)-(*RS*)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1*H*-1,2,4-triazol-1-yl)pent-1-en-3-ol
CAS name: (*E*)-($\pm$)-β-[(2,4-dichlorophenyl)methylene]-α-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol
CAS no.: 83657-24-3

Uses and mechanism

Diniconazole is a steroid methylation inhibitor and a systemic fungicide with protective and curative actions. It is used to control leaf and ear diseases in cereals, powdery mildew in vines, powdery mildew, rust and black spot in roses. It is also used on fruit, vegetables, and ornamentals. It is formulated as emulsifiable concentrate, suspension concentrate, water dispersible granules, and powder.

Toxicology

Acute toxicity

The rat oral LD_{50} is 639 mg/kg (males) and 474 mg/kg (females). The percutaneous rat LD_{50} is >5000 mg/kg, while the rat 4-h inhalation LC_{50} is >2770 mg/L.

Primary irritation

Diniconazole is not irritant to rabbit skin but is moderately irritating to rabbit eye.

Metabolism and toxicokinetics

By the peroral route, diniconazole is rapidly metabolized by hydroxylation of the *tert*-butyl methyl groups. By 7 days, 52–87 per cent is excreted in faeces and 13–46 per cent in urine (Tomlin, 2000).

Human and occupational toxicology

WHO toxicity class: III
EC hazard rating: Xn, R22, N, R50, R53

Environment and ecotoxicology

Avian acute oral LD_{50} values include: 1490 mg/kg for bobwhite quail and >2000 mg/kg for mallard duck. The 8-day dietary LC_{50} for mallard ducks is

5075 mg/kg (diet). Aquatic 96-h LC_{50} values include: 1.58 mg/L for rainbow trout, 6.84 mg/L for Japanese killifish, and 4.0 mg/L for carp. For bees, the acute contact LD_{50} is >20 μg/bee.

Etridazole

Chemical identification

Class: thiadiazole
Structural formula: see Figure 6.29
Molecular weight: 247.5
Common names: etridazole; ethazol; echlomezol; terrazole
IUPAC name: ethyl 3-trichloromethyl-1,2,4-thiadiazol-5-yl ether
CAS name: 5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole
CAS no.: 2593-15-9
EEC no.: 219-991-8

Uses and mechanism

Etridazole is a contact fungicide with protective and curative actions used to control *Phytophthoria* and *Pythium* in ornamentals, cotton, peanuts, vegetables, and turf. It is applied at rates of 0.15–0.4 lb/ha. It is available as dispersible powder, emulsion concentrate, and wettable powder.

Toxicology

Acute toxicity

The rat oral LD_{50} is 1100 mg/kg and signs include hyperactivity and ataxia. The percutaneous rabbit LD_{50} is >5000 mg/kg. The rat 4-h inhalation LC_{50} is >5.7 mg/L.

Primary irritation

Etridazole is not irritant to rabbit skin and is slightly irritant to rabbit eye.

Figure 6.29 Etridiazole

Short-term and subchronic toxicology

A 3-month dietary study with dogs at 1000 ppm showed decreased body weight gain, decreased relative spleen weight, increased liver weight, and increased serum liver enzymes (Borzelleca, Egle, and Hennigar, 1980).

Metabolism and toxicokinetics

After peroral dosing of etridazole in the rat, 3-carbonyl-5-ethoxy-1,2,4-thiadiazole was a major metabolite in urine and *N*-acetyl-5-(5-ethoxy-1,2,4-thiadazol-3-yl-methyl) L-cysteine a minor metabolite (Tomlin, 2000).

Human and occupational toxicology

ADI: 0.025 mg/kg
WHO toxicity class: III
EC hazard rating: R40, T, R23, Xn, R21/22, N, R50, R53

Environment and ecotoxicology

In plants, the trichloromethyl group is converted to the acid and alcohol, and the ethoxy group is hydroxylated to form a hydroxyethyl derivative. Soil DT_{50} (silt loam; 25°C) is 9.5 days (anaerobic) and 3 days (aerobic). Soil absorption is $K = 5.31$ (sandy). Avian oral LD_{50}s include: for bobwhite quail 560 mg/kg. Eight-day dietary LC_{50} are >5000 ppm for bobwhite quail and 1650 mg/kg for mallard duck. Toxicity values for aquatic species include:

Rainbow trout	216-h $LC_{50} = 1.21$ mg/L
Bluegill sunfish	216-h $LC_{50} = 3.27$ mg/L
Mysidopsis bahia	96-h $LC_{50} = 2.5$ mg/L
Crassostrea	96-h $LC_{50} = 3.0$ mg/L
Daphnia	48-h $LC_{50} = 4.9$ mg/L
Selenastrum	5-day $EC_{50} = 0.072$ mg/L
Anabaena	5-day $EC_{50} = 0.29$ mg/L
Naricula	5-day $EC_{50} = 0.43$ mg/L
Skeletonema	5-day $EC_{50} = 0.38$ mg/L

Fenbuconazole

Chemical identification

Class: triazole
Structural formula: see Figure 6.30

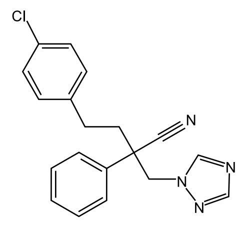


Figure 6.30 Fenbuconazole

Molecular weight: 336.8
Common name: fenbuconazole
IUPAC name: 4-(4-chlorophenyl)-2-phenyl-2(1*H*-1,2,4-trazol-1-ylmethyl)butyronitrile
CAS name: α-[2-(4-chlorophenyl)ethyl]-α-phenyl-1*H*-1,2,4-triazole-1-propanenitrile
CAS no.: 114369-43-6

Uses and mechanism

Fenbuconazole is a steroid demethylation inhibitor and a systemic fungicide with protective, curative, and eradicant functions. Fenbuconazole is used to control a wide range of diseases on field crops, rice, bananas, tree nuts, vegetables, and ornamentals as foliar, seed, and post-harvest treatments. Fenbuconazole is formulated as emulsifiable and suspension concentrates.

Toxicology

Acute toxicity

The rat peroral LD_{50} is >2000 mg/kg and the rat percutaneous LD_{50} is >5000 mg/kg. The rat 4-h inhalation LC_{50} is >5000 mg/kg.

Human and occupational toxicology

ADI (JMPR): 0.03 mg/kg
WHO toxicity class: II

Environment and ecotoxicology

Soil adsorption is $K_{oc} = 2100–9000$. In bobwhite quail the 8-day LC_{50} is 4050 mg/kg while in the mallard duck the 8-day dietary LC_{50} is 2110 mg/kg. Aquatic toxicity values include: for bluegill sunfish a 96-h LC_{50} of 0.68 mg/L. For bees, the 96-h LC_{50} for dust exposure is >0.29 mg/bee.

Hexaconazole

Chemical identification

Class: triazole
Structural formula: see Figure 6.31
Molecular weight: 314.2
Common name: hexaconazole
IUPAC name: (*RS*)-2-(2,4-dichlorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)hexanol-2-ol
CAS name: (±)-α-butyl-α-(2,4-dichlorophenyl)-1*H*-1,2,4-triazole-1-ethanol
CAS no.: 79983-71-4

Uses and mechanism

Hexaconazole is a steroid demethylation inhibitor and a systemic fungicide with curative and protective actions. It is used to control many pathogens, notably *Ascomycetes* and *Basidomycetes*, in vines, fruits, and nuts. Hexaconazole is formulated as oil miscible liquid, suspension concentrate, and water soluble granules.

Toxicology

Acute toxicity

The oral rat LD_{50} is 2189 mg/kg (males) and 6071 mg/kg (females).The percutaneous rat LD_{50} is >2000 mg/kg. The rat 4-h inhalation LC_{50} is >5.9 mg/L.

Primary irritation

Hexaconazole is not irritating to rabbit skin but is a mild irritant to rabbit eye.

Human and occupational toxicology

ADI (JMPR): 0.005 mg/kg
WHO toxicity class: III
EC hazard rating: Xi, R43, N, R51, R53

Figure 6.31 Hexaconazole

Environment and ecotoxicology

The acute oral LD_{50} for the mallard duck is >4000 mg/kg. In aquatic species the 96-h LC_{50} for rainbow trout is 3.4 mg/L, for mirror carp is 5.94 mg/L, and for sheepshead minnow is 5.4 mg/L. In *Daphnia* the 48-h LC_{50} is 2.9 mg/L. For bees, the acute oral and contact LD_{50} is >0.1 mg/bee. Worms have 14-day LC_{50} of 414 mg/kg.

Penconazole

Chemical identification

Class: triazole
Structural formula: see Figure 6.32
Molecular weight: 284.2
Common name: penconazole
IUPAC name: 1-(2,4-dichloro-β-propylphenethyl)-1*H*-1,2,4-triazole
CAS name: 1-[2-(2,4-dichlorophentyl)pentyl]-1*H*-1,2,4-triazole
CAS no.: 66246-88-6
EEC no.: 266-275-6

Uses and mechanism

Penconazole is a sterol demethylation inhibitor and is a systemic fungicide with protective and curative actions. It is used in the control of powdery mildew on pome fruit and scab. It is also used to control *Ascomycetes*, *Basidomycetes*, and *Deuteromycetes* on vines, fruits, vegetables, and ornamentals. Penconazole is formulated as emulsifiable concentrate, emulsion, and wettable powder.

Toxicology

Acute toxicity

The oral LD_{50} in rats is 2125 mg/kg and in mice is 2444 mg/kg. The percutaneous rat LD_{50} is >3000 mg/kg and the rat inhalation 4-h LC_{50} is >4000 mg/m^3.

Figure 6.32 Penconazole

Human and occupational toxicology

ADI (JMPR): 0.03 mg/kg
WHO toxicity class: III

Environment and ecotoxicology

Plant metabolism includes hydroxylation of the propyl side chain, conjugation to glucosides, and metabolism to triazolylalanine and triazoylacetic acid. The soil DT_{50} is 133–343 days. The photolysis DT_{50} is 4 days. In avian species, the 8-day oral LD_{50} for Japanese quail is 2424 mg/kg, for Peking duck is >3000 mg/kg, and for mallard duck is >1590 mg/kg. For the mallard duck, the 8-day LC_{50} is >5620 ppm and for the bobwhite quail >5620 ppm. In aquatic species 96-h LC_{50}s are for rainbow trout 1.7–4.3 mg/L, for carp 3.8–4.6 mg/L, and for bluegill sunfish 2.1–2.8 mg/L. For *Daphnia* the 48-h IC_{50} is 7–11 mg/L, for *Scenedesmus subspicatus* the 48-h IC_{50} is 3 mg/L, while for *Selenastrum capricornutam* the 5-day EC_{50} is 0.83 mg/L. For bees, the oral and contact LD_{50} values are >5 μg/bee. In worms the 14-day LC_{50} is >1000 mg/kg.

Tebuconazole

Chemical identification

Class: triazole
Structural formula: see Figure 6.33
Molecular weight: 307.8
Common name: tebuconazole
IUPAC name: (*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl) pentan-3-ol
CAS name: (±)-α-[2-(4-chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol
Synonyms: fentrazole; terbuconazole; terburazole; ethyltrianol
CAS no.: 107534-96-3
EEC no.: 403-640-2

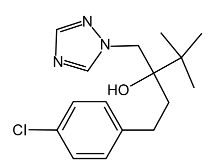


Figure 6.33 Tebuconazole

Uses and mechanism

Tebuconazole is a sterol demethylation inhibitor and a systemic fungicide with protective, curative, and eradicant actions. It is effective against a wide range of pathogenic fungi in fruit, vegetables, and ornamentals and is formulated as flowable concentrate, grease, seed treatment emulsion and gel, suspension concentrate, water dispersible granules, and powders.

Toxicology

Acute toxicity

The oral LD_{50} in the rat is 4000 mg/kg (males) and 1700 mg/kg (females) and in mice is 3000 mg/kg. The percutaneous rat LD_{50} is >5000 mg/kg and the rat 4-h inhalation LC_{50} is 0.37 mg/L (aerosol) and >5.1 mg/L (dust).

Primary irritation

Tebuconazole is not irritating to rabbit skin but slightly so to the rabbit eye.

Human and occupational toxicology

ADI (JMPR): 0.03 mg/kg
WHO toxicity class: III
EC hazard rating: Xn, R22

Environment and ecotoxicology

Avian acute toxicity values include for the Japanese quail an oral LD_{50} of 4438 mg/kg (males) and 2912 mg/kg (females) and for bobwhite quail 1988 mg/kg. For bobwhite quail, the 5-day dietary LC_{50} is > 5000 mg/kg (feed) and for mallard duck >4816 mg/kg (feed). For aquatic species the 96-h LC_{50} for rainbow trout is 4.4 mg/L and for bluegill sunfish 5.7 mg/L. For *Daphnia* the 48-h LC_{50} is 4.2 mg/L and for *Scenedesmus subspicatus* 4.01 mg/L. For bees, the 48-h peroral LD_{50} is 175.8 μg/bee and the contact LD_{50} is 0.6 μg/bee. For worms (*Eisenia foetidia*) the 14-day LC_{50} is 1381 mg/kg (soil).

Triadimefon

Chemical identification

Class: triazole
Structural formula: see Figure 6.34
Molecular weight: 293.8
Common name: triadimefon

Figure 6.34 Triadimefon

IUPAC name: 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-one
CAS name: 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanone
CAS no.: 43121-43-3
EEC no.: 256-103-8

Uses and mechanism

Triadimefon is a steroid demethylation inhibitor. It is a systemic fungicide with curative, protective, and eradicant properties, used to control powdery mildew on cereals, fruit, and vegetables. Triadimefon is available as dispersible powder, emulsifiable concentrate, water dispersible granules, and powder.

Toxicology

Acute toxicity

The oral LD_{50} in rats is 1000 mg/kg and dogs 500 mg/kg. The percutaneous rat LD_{50} is >5000 mg/kg. The rat 4-h inhalation LC_{50} is 3.27 mg/L (dust).

Primary irritation

Triadimefon is a mild irritant to skin and a moderate eye irritant in the rabbit.

Metabolism and toxicokinetics

Following peroral dosing of triadimefon, 83–96 per cent is excreted unchanged in urine and faeces within 2 to 3 days. Metabolism occurs in the liver mainly to triadimenol and its glucuronide conjugates. The $t_{1/2}$ in blood plasma is 2.5 h.

Human and occupational toxicology

ADI (JMPR): 0.03 mg/kg
WHO toxicity class: III
EC hazard rating: Xn, R22, N, R51, R53

Environment and ecotoxicology

In plants the carbonyl group is reduced to a hydroxyl group with the formation of triadimenol. A similar conversion occurs in soil with a DT_{50} of 18 days. $K_{oc} = 300$. Avian toxicity values include in mallard duck an acute oral LD_{50} of >4000 mg/kg. In the mallard duck, the 5-day dietary LC_{50} is >10 000 mg/kg (diet) and in bobwhite quail it is >4640 mg/kg. For aquatic species, toxicity values include 96-h LC_{50}s for bluegill sunfish of 11.0 mg/L, for orfe of 13.8 mg/L, and for rainbow trout of 17.4 mg/L. In *Daphnia* the 48-h LC_{50} is 11.3 mg/L and in *Scenedesmus subspicatus* the EC_{50} is 1.71 mg/L. For bees, the contact LD_{50} is >100 μg/bee.

Triadimenol

Chemical identification

Class: triazole
Structural Formula: see Figure 6.35
Molecular weight: 295.8
Common name: triadimenol
IUPAC name: (1*RS*,2*RS*,1*RS*,2*SR*)-1-(4-chlorophenoxy)–3,3-dimethyl–1-(1*H*-1,2,4-trazol-yl)butan-ol {Two diastereoisomers are A-(1*RS*,2*SR*) and B-(1*RS*,2*RS*); ratio is A:B 7:3}
CAS name: β-(4-chlorophenoxy)-α-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol
CAS no.: 55219-65-3
EEC no.: 259-537-6

Uses and mechanism

Triadimenol inhibits gibberellin and ergosterol synthesis and interferes with mitosis. It is a systemic fungicide with protective, curative, and eradicant effects. Triadimenol has extensive use against powdery mildews, rusts, and *Rhynchosporium* in a large variety of crops and is formulated as dispersible powder, emulsion, emulsion concentrate, flowable concentrate, granule, suspension concentrate, wettable granules, and powder.

Figure 6.35 Triadimenol

Toxicology

Acute toxicity

The oral LD_{50} in rats is 700 mg/kg and in mice is 1300 mg/kg. The percutaneous rat LD_{50} is >5000 mg/kg and the rat 4-h inhalation LC_{50} is >0.9 mg/L.

Primary irritation

Triadimenol is not irritating to rabbit skin or eyes.

Human and occupational toxicology

ADI (JMPR): 0.05 mg/kg
WHO toxicity class: III
EC hazard rating: Xn, R22

Environment and ecotoxicology

The soil DT_{50} is 110–375 days (sandy loam) and 240–270 days (loam). In avian species the acute oral LD_{50} in bobwhite quail is >2000 mg/kg. In aquatic species the 96-h LC_{50} in goldenorfe is 17.4–27.3 mg/L, in rainbow trout is 13–23.5 mg/L, and in bluegill sunfish is 15 mg/L. In *Daphnia* the 48-h LC_{50} is 51 mg/L. In *Scenedesmus subspicatus* the EC_{50} is 3.7 mg/L and in worms (*Eisenia foetidia*) the LC_{50} is 772 mg/kg (dry soil).

Morpholines

This group are derivatives of the basic morpholine (tetrahydro-1,4-oxazine) molecule. A major example is dodemorph and its acetate compound.

Dodemorph

Chemical identification

Dodemorph

Class: morpholine
Structural formula: see Figure 6.36
Molecular weight: 281.5
Common name: dodemorph
IUPAC and CAS name: 4-cyclododecyl-2,6-dimethylmorpholine

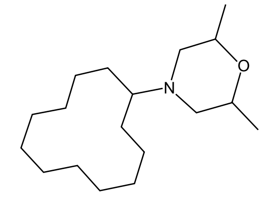


Figure 6.36 Dodemorph

CAS no.: 1593-77-7
EEC no.: 216-474-9

Dodemorph acetate

IUPAC name: *N*-cyclododecyl-2,6-dimethylmorphololium acetate
CAS no.: 31717-87-0

Uses and mechanism

Dodemorph is an inhibitor of ergosterol biosynthesis and is a systemic fungicide with protective and curative actions. It is absorbed through roots and leaves and is used to control powdery mildews on roses and other ornamentals. Dodemorph is formulated as an emulsifiable concentrate.

Toxicology

Acute toxicity

For dodemorph acetate the oral rat LD_{50} is 3944 mg/kg (males) and 2465 mg/kg (females). The percutaneous rat LD_{50} is >4000 mg/kg. The rat 4-h inhalation LC_{50} is 5 mg/L.

Primary irritation

Dodemorph acetate is moderately irritating to rabbit skin and severely irritating to rabbit eye.

Environment and ecotoxicology

For dodemorph acetate the soil DT_{50} is 73 days. $K_{oc} = 4200–48\,000$ (high adsorption). Aquatic toxicity values include for guppy a 96-h LC_{50} of 40 mg/L. In *Daphnia* the 48-h LC_{50} is 3.34 mg/L.

Fenpropimorph

Chemical identification

Class: morpholine
Structural formula: see Figure 6.37
Molecular weight: 303.5
Common name: fenpropimorph
IUPAC name: (±)-*cis*-4-[3-(4-*tert*-butylphenyl)2-methylpropyl]-2,6-dimethylmorpholine
CAS name: *cis*-4-[3-[4-(1,1-dimethylethyl)phenyl]-2-methylpropyl]-2,6-dimethylmorpholine
CAS no.: 67564-91-4
EEC no.: 266-719-9

Uses and mechanism

Fenpropimorph is an ergosterol biosynthesis inhibitor [by steroid reduction (sterol-Δ^{14}-reductase) and isomerization (Δ^8- to Δ^7-isomerase)]. Fenpropimorph is a systemic foliar fungicide with protective and curative effects. Xylem translocation occurs acropetally. Fenpropimorph is used to control several fungal species in cereals, sugar beet, beans, leeks and sunflowers. It is formulated as emulsifiable and suspension concentrates.

Toxicology

Acute toxicity

In the rat the oral LD_{50} is >3000 mg/kg, the percutaneous LD_{50} is >4000 mg/kg, and the 4-h inhalation LC_{50} is 3580 mg/m^3.

Primary irritation

Fenpropimorph is a mild irritant to rabbit skin.

N
O

Figure 6.37 Fenpropimorph

Human and occupational toxicology

ADI (JMPR): 0.003 mg/kg
WHO toxicity class: III
EC hazard rating: Xn, R20, Xi, R38, N, R51, R53

Environment and ecotoxicology

Fenpropimorph is strongly adsorbed to soil with a K_d of 22.6 (sand) and 73.9 (loamy sand). $K_{oc} = 2772–5943$. In avian species the acute peroral LD_{50} is 3900 mg/kg and the 5-day LC_{50} is 5000 mg/kg for mallard duck. In pheasants the oral LD_{50} is 3900 mg/kg. In bobwhite quail the 5-day LC_{50} is >5000 mg/kg. For aquatic species, 96-h LC_{50}s are: for rainbow trout 9.5 mg/L, for bluegill sunfish 3.2–4.6 mg/L, and for carp 3.2 mg/L. For *Daphnia* the 48-h LC_{50} is 2.4 mg/L, for *Chlorella fusca* the 96-h EC_{50} is 2.21 mg/L, and for *Pseudomonas putida* the 17-h EC_{10} is >1874 mg/L. The bee acute oral LD_{50} is >100 μg/bee. For earthworms, the 14-day LD_{50} is 562 mg/kg.

Tridemorph

Chemical identification

Class: morpholine
Structural formula: see Figure 6.38
Molecular weight: 297.5
Common name: tridemorph
IUPAC name: 4-alkyl-2,6-dimethyl morpholine (originally 2,6-dimethyl-4-tridecyl-morpholine)
CAS name: 2,6-dimethyl-4-tridecylmorpholine
CAS no.: 81412-43-3(original); 24602-86-6 (tridecyl-)
EEC no.: 246-347-3 (tridecyl-)

Composition: tridemorph was originally considered to consist of tridecyl C_{13} isomers but is now believed to be composed of C_{11} to C_{14} homologues containing

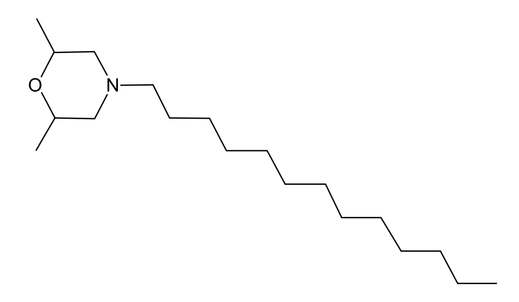


Figure 6.38 Tridemorph

60–70 per cent 4-tridecyl isomers, 0.2 per cent C_9 and C_{15} homologues, and 5 per cent 2,5-dimethyl isomers.

Uses and mechanism

Tridemorph is an ergosterol biosynthesis inhibitor, acting by inhibition of steroid reduction (sterol-Δ^{14}-reductase) and isomerization (Δ^8- to Δ^7-isomerase). It is a systemic fungicide with eradicant action. Tridemorph is absorbed by leaves and roots with protective action and is used to control *Erysiphe graminis* in cereals, *Mycosphaerella* in bananas, *Corticum salmonicolor* and *Exobasidium vexams* in tea, and *Odium heveae* in *Hevea*. It is formulated as an emulsifiable concentrate.

Toxicology

Acute toxicity

In rats, the oral LD_{50} is 480 mg/kg, the percutaneous LD_{50} is >4000 mg/kg, and the 4-h inhalation LC_{50} is 4.5 mg/L.

Primary irritation

Tridemorph is not irritant to rabbit skin or eyes.

Human and occupational toxicology

ADI: 0.016 mg/kg
WHO toxicity class: II
EC hazard rating: Xn, R20/22, Xi, R38, N, R50, R53

Environment and ecotoxicology

Metabolism in plants is by oxidation of the 4-alkyl side chain or opening of the morpholine ring. The soil DT_{50} is 14–34 days (field). $K_{oc} = 2500–10\,000$. The LD_{50} is 1388 mg/kg in quail and >2000 mg/kg in the duck. In trout the 96-h LC_{50} is 3.4 mg/L and in *Daphnia* the 48-h LC_{50} is 1.3 mg/L. Bees have a 24-h LD_{50} of >200 μg/bee. In worms (*Eisenia foetidia*) the14-day LD_{50} is 880 mg/kg.

Carboxanilides (oxathiins)

Carboxin

Chemical identification

Class: oxathiin
Structural formula: see Figure 6.39

Figure 6.39 Carboxin

Molecular weight: 235.3
Common name: carboxin; carbathiin
IUPAC name: 5,6-dihydro-2-methyl-1,4-oxathi-ine-3-carboxanilide
CAS name: 5,6-dihydro-2-methyl-*N*-phenyl-1,4-oxathiin-3-carboxamide
CAS no.: 5234-68-4

Uses and mechanism

Carboxin is a systemic fungicide. It is a seed treatment for the control of smuts and bunts. Carboxin is used to control *Rhizoctonia* in barley, wheat, oats, rice, cotton, nuts, and vegetables. It is formulated as suspension concentrate, flowable concentrate, and water dispersible powder.

Toxicology

Acute toxicity

The rat oral LD_{50} is 2000–3820 mg/kg. The percutaneous rabbit LD_{50} is >4000 mg/kg. The rat 1-h inhalation LD_{50} is >20 mg/L.

Primary irritation

Carboxin is moderately irritating to the rabbit eye.

Metabolism and toxicokinetics

In rat and rabbit the principal metabolic pathway is *o*- or *p*-hydroxylation, followed by glucuronidation.

Human and occupational toxicology

Ingestion may produce nausea, vomiting, and headache.

ADI: 0.01 mg/kg
WHO toxicity class: III

Environment and ecotoxicology

Plant metabolism involves oxidation to the sulphoxide. The soil DT_{50} is 24 h. $K_{oc} = 373$. In the mallard duck the 8-day dietary LD_{50} is >4640 mg/kg and in bobwhite quail is >10 000 mg/kg. For rainbow trout the 96-h LC_{50} is 2.0 mg/L and for bluegill sunfish it is 1.2 mg/L. For *Daphnia* the 48-h LC_{50} is 84.4 mg/L, for *Chlorella* the 96-h EC_{50} is 2.4 mg/L, for *Selenastrum* the 96-h EC_{50} is 0.48 mg/L and for *Lemna* the 14-day EC_{50} is 0.92 mg/L. Bees have an LD_{50} of >181 μg/bee. For earthworms, the 14-day LC_{50} is 500–1000 ppm.

Oxycarboxin

Chemical identification

Class: oxathiin
Structural formula: see Figure 6.40
Molecular weight: 267.3
Common name: oxycarboxin
IUPAC name: 5,6-dihydro-2-methyl-1,4-oxathi-ine-3carboxanilide 4,4-dioxide
CAS name: 5,6-dihydro-2-methyl-*N*-phenyl-1,4-oxathiin-3-carboxamide 4,4-dioxide
CAS no.: 5259-88-1
EEC no.: 226-066-2

Uses and mechanism

Oxycarboxin is a systemic fungicide with curative action. It is used by foliar application to control rust diseases on ornamentals and cereals. It is formulated as emulsifiable concentrate and wettable powder.

Toxicology

Acute toxicity

The oral rat LD_{50} is 5816 mg/kg (males) and 1632 mg/kg (females). The percutaneous rabbit LD_{50} is >5000 mg/kg. The rat 4-h inhalation LC_{50} is >5000 mg/L.

Figure 6.40 Oxycarboxin

Primary irritation

Oxycarboxin is not irritant to rabbit skin, but is mildly irritant to rabbit eye.

Human and occupational toxicology

ADI: 0.15 mg/kg
WHO toxicity class: III
EC hazard rating: Xn, R22, R52, R53

Environment and ecotoxicology

The soil DT_{50} is 2.5–8 weeks (sandy loam). In mallard duck the LD_{50} is 1250 mg/kg and the 8-day dietary LC_{50} is >4640 ppm. In bobwhite quail, the 8-day LC_{50} is >10 000 ppm. In rainbow trout the 96-h LC_{50} is 19.9 mg/L and in bluegill sunfish 28.1 mg/L. In *Daphnia* the 48-h LC_{50} is 69.1 mg/L, while in *Chlorella* the 96-h LC_{50} is 19 mg/L. In bees, the contact LD_{50} is >181 μg/bee.

Organophosphates

Pyrazophos

Chemical identification

Class: phosphorothiolate
Structural formula: see Figure 6.41
Molecular weight: 373.4
Common name: pyrazophos
IUPAC name: ethyl 2-diethoxyphosphinothioyloxy-5-methylpyrazolol[1,5-a]pyramidine-6-carboxylate; *O*,*O*-diethyl-*O*-6-ethoxycarboyl-5-methylpyrazolol[1,5-a]pymidim-2-yl phosphorothiolate
CAS name: ethyl 2-[(diethoxyphosphinothioyl)oxy]-5-methylpyrazolo[1,5-a]pyrimidine-6-carboxylate
CAS no.: 13457-18-6
EEC no.: 236-656-1

Figure 6.41 Pyrazophos

Uses and mechanism

Pyrazophos is a systemic fungicide with protective and curative actions. It is absorbed by leaves and shoots, and transported acropetally. It is used to control many fungi in various fruit and vegetables and is formulated as an emulsifiable concentrate and wettable powder.

Toxicity

Acute toxicity

The rat oral LD_{50} is 151–778 mg/kg, the percutaneous LD_{50} is $>$2000 mg/kg, and the 4-h inhalation LC_{50} is 1220 mg/m^3.

Primary irritation

Pyrazophos is not irritating to rabbit skin but is slightly irritating to rabbit eye.

Human and occupational toxicology

ADI (JMPR): 0.004 mg/kg
WHO toxicity class: II
EC hazard rating: Xn, R22

Environment and ecotoxicology

Degradation in soil occurs by cleavage of the phosphoric acid group, saponification of the carboxylate fraction, and degradation of the heterocyclic ring. The DT_{50} is 10–21 days, and the DT_{90} is 111–235 days. $K_{oc} = 1332–2670$. In mallard duck the 14-day dietary LC_{50} is 340 mg/kg, while in bobwhite quail it is 300 mg/kg. In aquatic species the 96-h LC_{50} for rainbow trout is 0.48–1.14 mg/L, for carp 2.8–6.1 mg/L, and for bluegill sunfish 0.28 mg/L. In *Scenedesmus subspicatus* the 72-h LC_{50} is 65.5 mg/L, while in bees the 72-h contact LC_{50} is 0.25 μg/bee. For worms (*Eisena foetidia*) the14-day LC_{50} is $>$1000 mg/kg (soil).

Tolclofos-methyl

Chemical identification

Class: phosphorothiolate
Structural formula: see Figure 6.42
Molecular weight: 301.1
Common name: tolclofos-methyl
IUPAC name: *O*-2,6-dichloro-*p*-tolyl *O*,*O*-dimethyl phosphorothiolate

Figure 6.42 Tolclofos-methyl

CAS name: *O*-(2,6-dichloro-4-methyl phenyl) *O*,*O*-dimethyl phosphorothioate
CAS no.: 570018-04-9

Uses and mechanism

Tolclofos-methyl inhibits phospholipid synthesis, leading to cessation of germination of spores and growth of fungal mycelium. It is a contact fungicide with protective and curative actions, used to control *Rhizoctonia*, *Corticum*, *Sclerotium*, and *Typhula* on potatoes, sugar beet, cotton, peanuts, vegetables, cereals, ornamentals, and lawn turf. It is formulated as dispersible power, emulsion and suspension concentrates, and wettable powder.

Toxicology

Acute toxicity

The rat oral LD_{50} is $>5000\,mg/kg$, the rat percutaneous LD_{50} is $>5000\,mg/kg$, and the rat inhalation LC_{50} is $>3320\,mg/m^3$.

Primary irritation

Tolclofos-methyl is not irritating to rabbit skin but is a slight irritant to rabbit eye.

Metabolism and toxicokinetics

Tolclofos-methyl is rapidly metabolized by oxidative desulphuration of the P═S group to a P═O group, oxidation at the 4-methyl group, and cleavage of the P—O aryl and P—O methyl links.

Human and occupational toxicology

ADI (JMPR): 0.07 mg/kg
WHO toxicity class: III

Environment and ecotoxicology

The DT_{50} is 44 days (water), 15–28 days (river water), and <2 days (soil surface). The acute oral LD_{50} is >5000 mg/kg in both the mallard duck and bobwhite quail. In bluegill sunfish the 96-h LC_{50} is >720 mg/L.

Piperazines

Triforine

Chemical identification

Class: piperazine
Structural formula: see Figure 6.43
Molecular weight: 435
Common name: triforine
IUPAC name: *N*,*N*′-[piperazine-1,4-diylbis[(trichloromethyl)methylene]]diformamide; 1,1′-piperidine-1,4-diyldi-[*n*-(2,2,2-trichloromethyl)formamide]
CAS name: *N*,*N*′-[1,4-piperazinediylbis(2,2,2-trichloroethylidene)]bis formamide
CAS no.: 26644-46-2

Uses and mechanism

Triforine is an inhibitor of ergosterol biosynthesis and is a systemic fungicide with protective and curative actions. It is absorbed by leaves and roots with acropetal translocation and is used to control mildew, rust, rots, and scabs in cereals, fruits, hops, ornamentals, and some vegetables. It is also used to control *Monilia* on stone fruit and *Ascochyta* on chrysanthemums.

Figure 6.43 Triforine

Toxicology

Acute toxicity

The oral LD_{50} in rats and mice is >10 000 mg/kg and in dogs >2000 mg/kg. The intraperitoneal rat LD_{50} is >4000 mg/kg, the percutaneous rabbit LD_{50} is >10 000 mg/kg, and the rat 4-h inhalation LC_{50} is >4.5 mg/L.

Human and occupational toxicology

ADI (JMPR): 0.02 mg/kg
WHO toxicity class: III

Environment and ecotoxicology

The soil DT_{50} is 3 weeks. It does not accumulate in the environment. The oral LD_{50} for bobwhite quail is >5000 mg/kg. For bluegill sunfish and rainbow trout the 96-h LC_{50} is >1000 mg/L. In *Daphnia* the 48-h LC_{50} = 117 mg/L and in *Scenedesmus capricornutam* the EC_{50} is 380 mg/L.

Metallic fungicides

Inorganic metallic fungicides

These were some of the first fungicides used in agriculture, particularly those listed in Table 6.2. In general inorganic fungicides are protective or preventative.

Table 6.2 Inorganic metallic fungicides

Cadmium chloride
Cadmium sulphate
Copper acetate
Copper ammonium carbonate
Copper carbonate
Copper chloride
Copper hydroxide
Copper oxychloride
Copper silicate
Copper sulphate
Cupric oxide
Cuprous oxide
Mercuric chloride
Mercuric oxide
Mercury sublimate

The mercuric and mercurous compounds have been withdrawn in many countries because of their adverse toxicity and environmental effects.

Organometallic fungicides

These materials are both aliphatic and aromatic (Table 6.3). Many are of moderate to high mammalian and human toxicity, several being immunotoxic and neurotoxic. Typical organotins are di- and tri-alkyl and triphenyl tins. In general they are severely irritant to the skin, eye, and muscosae, and several are hepatorenotoxic and immunotoxic. For example, they have been shown to increase susceptibility to infections, decrease lymphopoiesis, and decrease T-lymphocyte production (Descotes, 1986; Verschurren *et al.*, 1966). Tri-alkyl tins cross the blood–brain barrier and are centrally neurotoxic. In humans they may cause increased intracranial pressure, headache, vertigo, photophobia, blurred vision, nausea, vomiting, disorientation, amnesia, tremors, convulsions, aggressive behaviour and other neurobehavioural disturbances, muscle weakness, and paralysis (Phillips, 2001). Organotin compounds, particularly triethyltin, uncouple oxidative phosphorylation

Table 6.3 Organometallic fungicides

Bistributyltin oxide
Cadmium succinate
Copper acetate
Copper linoleate
Copper naphtholate
Copper oleate
Copper phenyl salicylate
Copper quinolinolate
Copper resinate
Fenbutin oxide
Mercury acetate
Mercury benzoate
Mercury pentachorophenate
Mercury propionate
Mercury quinolinate
Methoxyethyl mercury acetate
Methoxyethyl mercury chloride
Methylmercury hydroxide
Methylmercury nitrile
Phenylmercuric acetate
Tributyltin
Triethyltin
Trimethyltin
Triphenyltin (fentin hydroxide)

(Stockdale and Selwyn, 1971). The high toxicity of organotin compounds is reflected in their low occupational exposure limits: $TWA_8 = 0.1\,mg/m^3$, STEL $0.2\,mg/m^3$ (ACGIH, 2003). Detailed reviews can be found in Kaloyanova and Batawi (1991) and WHO (1980, 1990a).

Organomercurials were used for post-harvesting application to grain and other seed crops. However, a large poisoning epidemic in Iraq following the consumption of treated seeds led to a ban on their use in the mid-1970s (Bakir, Damlaji, and Amin-Zaki, 1973). Like organotin compounds, organomercurials are notoriously neurotoxic. ACGIH workplace exposure guidelines are TWA_8 $0.01\,mg/m^3$ (STEL $0.03\,mg/m^3$) for alkyl compounds and TWA_8 $0.1\,mg/m^3$ for aryl compounds (ACGIH, 2003). Organomercurials have been reviewed by Ecobichon (1982) and WHO (1990b).

Miscellaneous

Several antifungal substances do not readily fit into classical classifications because they are isolated materials or are classified under other (non-antifungal) uses and/or mechanisms. Included in this miscellaneous group are aliphatic aldehydes, antibiotic substances, thiocarbonates, and cinnamic acid derivatives. The essentials are reviewed briefly.

Aliphatic aldehydes

Several aliphatic aldehydes are used as fungicides, amongst them formaldehyde and formaldehyde releasers, which have been considered in detail elsewhere (Clary, Gibson, and Waritz, 1983; Feinman, 1988). One aldehyde of considerable toxicological interest is acrolein, which is considered in detail below.

Acrolein

Chemical identification

Class: aliphatic aldehyde
Structural formula: see Figure 6.44
Molecular weight: 56.1

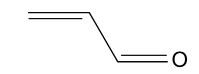


Figure 6.44 Acrolein

Common name: acrolein
IUPAC name: prop-2-enal; acrylaldehyde
CAS name: 2-propenal
Synonyms: allyl aldehyde; acraldehyde
CAS no.: 107-02-8
EEC no.: 202-453-4

Uses and mechanism

Acrolein reacts with SH groups. It is formulated as a liquid.

Toxicology

Acute toxicity

The rat oral LD_{50} is 29 mg/kg and the mouse oral LD_{50} is 13.9 mg/kg (males) and 17.7 mg/kg (females). The percutaneous rabbit LD_{50} is 231 mg/kg. By inhalation the rat 1-h LC_{50} is 65 mg/m^3 (males) and 60 mg/m^3 (females): the rat 4-h LC_{50} is 18.5 mg/m^3 (males) and 22 mg/m^3 (females). The rat 30-min LC_{50} is 131 ppm and the 10-min LC_{50} is 355 ppm (Ballantyne *et al.*, 1989; Catalina, Thieblot, and Champery, 1966; EPA, 1998; Skog, 1950). RD_{50} values range from 1.2 to 6.6 mg/m^3 (Tsubone and Kawata, 1991; WHO, 1992).

Short-term and subchronic toxicology

Hamsters, rats, and rabbits were exposed to acrolein vapour at 0, 0.4, 1.4, and 4.9 ppm for 6 h/day for 5 days/week for 13 weeks. At 4.9 ppm there was mortality, ocular and nasal irritation, depression of growth, and inflammation, necrosis, hyperplasia, and metaplasia of the respiratory tract epithelium. A no-effect concentration was not established for the rat (Feron *et al.*, 1978).

Chronic toxicology and oncogenicity

Sprague-Dawley rats were given acrolein by gavage at daily dosages of 0, 0.05, 0.5, and 2.5 mg/kg up to 102 weeks. The only effects noted were decreased serum creatinine kinase and increased early cumulative mortality. There were no significant increases in neoplastic or non-neoplastic histopathology (Parent, Caravello, and Long, 1992a). Given by intraperitoneal injection to male Fischer 344 rats, acrolein had an initiating activity for urinary bladder carcinogenesis (Cohen *et al.*, 1992).

Genetic toxicology

In reverse mutation assays acrolein was positive in *Salmonella typhimurium* in strains TA98, TA100, TA104, and TA1535 and in *Escherichia coli* WP2 uvrA. It was

also positive in *Saccharomyces cerevisiae*. Forward mutation tests were conducted in some laboratories but not in others. Mutations were found with hamster V79 cells, and in *Drosophila melanogaster* young larvae but not adults. Chromosome aberrations were seen in CHO cells *in vitro*, sister chromatid aberrations in human lymphocytes *in vitro*, but not in dominant lethal mutations in mouse germ cells. Negative results were obtained with a rat bone marrow cytogenetics study, *in vitro* chromosomal aberration and sister chromatid exchange studies in CHO cells, and a forward gene mutation study in CHO cells (HGPRT locus) (Parent, Caravello, and Harbell, 1991). Genotoxicity has been reviewed in detail by WHO (1992).

Developmental toxicology

Acrolein was embryotoxic and teratogenic *in vivo* in rats and rabbits when injected into the amniotic sac (Claussen, Hellman, and Pache, 1980; Hales, 1982). However, *in vitro* studies in rat whole embryo cultures do not show teratogenic effects (Mirkes *et al.*, 1981, 1984; Schmid *et al.*, 1981). In order to examine the reasons for the discrepancy between the *in vivo* and *in vitro* studies, Slott and Hales (1986) further examined the response of the cultured rat embryo preparation, but over a narrower concentration range. They found acrolein to be both embryolethal and teratogenic, with the EC_{50} for embryo malformations being higher than that for embryomortality, indicating a narrow teratogenic range. With a serum medium the EC_{50}s for malformations and lethalities were 137 and 115 μM, respectively, and for the serum-free medium, 2.8 and 8.3 μM. Also, the slope of the acrolein log concentration–response curve for the serum-free medium was twice that for the serum medium, indicating there may be different mechanisms of action in the two media, possibly in the extent or specificity of acrolein binding to embryonic macromolecules or differing capacities of glutathione (or other) protective mechanisms. Chhibber and Gilani (1896) confirmed that acrolein is embryotoxic at higher doses and moderately teratogenic in chick embryos. New Zealand White rabbits given acrolein by gavage at 0, 0.1, 0.75, or 2.0 $mg\,kg^{-1}\,day^{-1}$ over gd 7–19 showed maternal toxicity (mortality, food consumption, and body weight) and increased resorptions at the high dose, but not with statistical significance. Acrolein was not embryofetotoxic or teratogenic under the conditions of the study (Parent *et al.*, 1993a).

Reproductive toxicology

Male and female rats were incubated and given 70 daily doses of acrolein at 0, 1, 3, or 6 mk/kg. The F_0 generation was assigned to a 21-day period of co-habitation and dosing of females continued through co-habitation, gestation, and lactation. F_1 generation pups were similarly treated. In general, reproductive indices were unaffected, with the exception of reduced pup weights in the F_1 generation at the high dose. Gastric lesions were consistently found in the high dose and some mid-dose animals; erosions of the glandular mucosa and hyperplasia/hyperkeratosis of the forestomach were the

most frequent lesions. Relative to the controls, mortality and body weight gain decreases were noted for high dosage animals (Parent, Caravello, and Hiberman, 1992b).

Metabolism and toxicokinetics

Two major detoxification pathways are conjugation with glutathione and oxidation to acrylic acid. In the rat, a major metabolite is 3-hydroxypropylmercapturic acid and a minor metabolite is 2-carboxyethylmercapturic acid (Linhart *et al.*, 1961). After oral or intravenous dosing with [α,β-C^{14}]-acrolein routes of excretion were urine (up to 69 per cent), expired air (up to 30 per cent mainly as $^{14}CO_2$) and faeces (up to 30 per cent after oral dosing). Urine metabolites included S-2-carboxyethylmercapturic acid (about 34 per cent) and S-3-hydroxypropylmercapturic acid (about 7 per cent) (Parent *et al.*, 1993b; Parent, Caravello, and Sharp, 1996).

Human and occupational toxicology

Acrolein vapour is highly irritating to skin, eyes, and respiratory tract. Irritation thresholds are 0.13 mg/m^3 for eye (including blepharospasm) and nose, and 0.7 mg/m^3 for depression of respiratory rate. Skin contact with liquid acrolein causes erythema and oedema, and splash contamination of the eye causes blepharoconjunctivitis, oedema of the lids, and corneal injury (Grant and Schuman, 1993). Acrolein causes injury to the plasma membrane of vascular endothelial cells, accompanied by reductions in glutathione and protein SH groups (Patel and Block, 1990). Typical pulmonary damage includes congestion, bronchiolar epithelial necrosis and sloughing, alveolar haemorrhages, and oedema (Ballantyne *et al.*, 1989; Kutzman *et al.*, 1985). Inhalation of vapour may cause clinical pulmonary oedema. Post-exposure bronchiectasis has been described (Mahut e*t al.*, 1993). Short-term exposures as low as 10 ppm may be lethal. Acrolein suppresses respiratory host defence against infections. It causes a dose-dependant cytotoxicity to pulmonary alveolar cells (aptosis and necrosis), and a dose-dependant inhibition of release of IL-1β, TNF-α, and IL-12. The inhibition of cytokine release and cytotoxicity to alveolar macrophages may be a cause for acrolein-induced pulmonary immunosuppression (Li *et al.*, 1997).

WHO toxicity class: Ia
EC hazard rating: F, R11, T+, R26, T, R25, C, R34
ACGIH assessment: TWA = 0.1 ppm (ceiling); skin, A4 notation (ACGIH, 2003)
IARC assessment: group 3
Acute exposure guideline levels (AEGL; EPA, 1998):
AEGL-1 (not disabling); 30-min, 1-, 4-, and 8-h – all 0.03 ppm
AEGL-2 (disabling); 30-min, 1-h 0.2 ppm; 4- and 8-h 0.1 ppm
AEGL (lethality); 30-min 1.98 ppm; 1-h 1.0 ppm; 4-h 0.07 ppm; 8-h 0.50 ppm

Environment and ecotoxicology

The DT_{50}s (water) are 150 h (pH 5), 120–180 h (pH 7), and 5–40 h (pH 9). The field dissipation DT_{50} is 7.5–10.2 h. Avian toxicity values include:

Bobwhite quail	acute peroral $LD_{50} = 19$ mg/kg
Mallard duck	acute peroral $LD_{50} = 30.2$ mg/kg

Aquatic toxicity values include:

Rainbow trout	24-h $LC_{50} = 0.15$ mg/L
Bluegill sunfish	24-h $LC_{50} = 0.079$ mg/L
Shiners	24-h $LC_{50} = 0.04$ mg/L
Rasbora heteromorpha	24-h $LC_{50} = 0.39$ mg/L
Pimephales promelas	144-h $LC_{50} = 0.084$ mg/L
Leuciscus idus melanotus	48-h $LC_{50} = 0.25$ mg/L
Oncorhynchus kisutch	96-h $LC_{50} = 0.068$ mg/L
Shrimp	48-h $LC_{50} = 0.1$ mg/L
Oyster	48-h $LC_{50} = 0.46$ mg/L
Lemna gibba	14-day $EC_{50} = 0.07$ mg/L
Daphnia magna	48-h $LC_{50} = 0.057$ mg/L
Selenastrum capricornutam	5-day $EC_{50} = 0.03$ mg/L
Anabaena flos-aquae	5-day $EC_{50} = 0.042$ mg/L
Navicula pelliculosa	5-day $EC_{50} = 0.07$ mg/L
Skeletonema costatum	5-day $EC_{50} = 0.03$ mg/L

Acrolein has been reviewed in detail by ATSDR (1990), Beauchamp *et al.* (1985), and WHO (1992).

Thiocarbonates

Sodium tetrathiocarbonate

Chemical identification

Class: thiocarbonate
Structural formula: see Figure 6.45
Molecular weight: 186.2
Common name: sodium tetrathiocarbonate
IUPAC name: sodium tetrathio(peroxocarbonate)
CAS name: disodium carbono(dithioperoxodithioate)
Synonym: GY-81
CAS no.: 7345-69-9

Figure 6.45 Sodium tetrathiocarbonate

Uses and mechanism

Soil degradation to carbon disulphide takes place. It is a contact fungicide for the control of various soil pathogens causing root rot and is formulated as a soluble concentrate.

Toxicology

Acute toxicity

The rat oral LD_{50} is 631 mg/kg, the percutaneous LD_{50} in rabbits is >2000 mg/kg, and the rat 4-h inhalation LC_{50} values are 4.73 mg/L (males) and 3.17 mg/L (females).

Primary irritation

Sodium tetrathiocarbonate is a moderately severe irritant to rabbit skin and a marked irritant to rabbit eye.

Environment and ecotoxicology

There is rapid degradation in soil releasing carbon disulphide. The oral LD_{50} in bobwhite quail is 1180 mg/kg, while the 5-day LC_{50} is >5620 mg/kg in both bobwhite quail and mallard duck. In rainbow trout the 96-h LC_{50} is 6.7 mg/L and in bluegill sunfish 21 mg/L. In *Daphnia* the 48-h $LC_{50} = 6.6$ mg/L.

Antibiotics

Antifungal antibiotics range from relatively simple substances such as penicillamine (α-amino-β-methylmercaptobutyric acid) to complex multi-ring structures, and hence have widely varying toxicity. For example, the rat acute peroral LD_{50} of blasticidin-S is 16 mg/kg, whereas that of validomycin is >20 000 mg/kg (Edwards, Ferry, and Temple, 1991). Some have potentially severe toxicity for humans, e.g. streptomycin causes vestibular nerve damage and aplastic anaemia. In contrast, blasticidin-S, of greater acute toxicity, is associated with a lesser degree

of long-term toxicity (conjunctivitis, dermatitis, and reversible irritant effects) (Edwards, Ferry, and Temple, 1991). Antibiotics with fungicidal properties include cycloheximide, iturin A, validomycin, streptomycin, and griseofulvin.

Iturin-A, a cyclic lipopeptide produced by *Bacillus subtilis*, has strong activity against microflora in seeds. Griseofulvin is used both as an agricultural fungicide and a systemic treatment for fungal infections in humans.

Cycloheximide

Chemical identification

Class: glutarimide
Structural formula: see Figure 6.46
Molecular weight: 281.17
Common name: cycloheximide
IUPAC name: 4-[(2*R*)-2-[(1*S*,3*S*,5*S*)-(3,5-dimethyl-2-oxocyclohexl)]-2-hydroxyethyl]piperidine-2,6-dione; 3-[(2*R*)-2-[(1*S*,3*S*,5*S*)-3,5-dimethyl-2-oxocyclohexyl]-2-hydroxyethyl]glutarimide
CAS name: [1*S*-[1(*S*′),3,5]]-4-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-2,6-piperidine dione
Synonym: 3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide; actidione; isocycloheximide; naramycid
CAS no.: 66-81-9

Uses and mechanism

Cycloheximide is an inhibitor of protein synthesis and is available as a wettable powder.

Toxicology

Acute toxicity

The oral rat LD_{50} is 2 mg/kg.

Figure 6.46 Cycloheximide

Genetic toxicology

Cycloheximide is mutagenic in *Escherichia coli* with metabolic activation, and positive in a mouse sperm morphology assay (Hathaway *et al*., 1993).

Developmental toxicology

Given to pregnant rats by intraperitoneal injection at 250 g/kg on gd 10, cycloheximide produced central nervous system, craniofacial, and cardiovascular abnormalities. Mice given 30 mg/kg on gd 9 produced musculoskeletal abnormalities (Hathaway *et al*., 1993).

Human and occupational toxicology

The estimated lethal oral dose is 5–50 mg/kg (Hathaway *et al*., 1993).

Cinnamic acid class

Dimethomorph

Chemical identification

Class: cinnamic acid
Structural formula: see Figure 6.47
Molecular weight: 387.9
Common name: dimethomorph
IUPAC name: (*E*,*Z*)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)acryoyl]morpholine
CAS name: (*E*,*Z*)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-1-oxo-2-propenyl]morpholine
CAS no.: 110488-70-5
EEC no.: 404-200-2

O
NH
O
O
O
Cl

Figure 6.47 Dimethomorph

Uses and mechanism

Dimethomorph inhibits the formation of oomycete fungal cell wall. It is a local fungicide with protectant and antisporulent activities, effective against *Peronosporaceae* and *Phytophthora* in various crops. Dimethomorph is formulated as dispersible concentrate, water dispersible granules, and wettable powders.

Toxicology

Acute toxicity

Oral LD_{50}s are: for male rats 4300 mg/kg, for female rats 3500 mg/kg, for male mice >5000 mg/kg, and for female mice 3700 mg/kg. The rat intraperitoneal LD_{50} is 327 mg/kg (males) 297 mg/kg (females). The percutaneous rat LD_{50} is >5000 mg/kg, while the rat 4-h inhalation $LC_{50} > 4.2$ mg/L.

Primary irritation

Dimethomorph is not irritant to rabbit skin and minimally irritant to rabbit eye.

Human and occupational toxicology

ADI: 0.09 mg/kg
WHO toxicity class: III

Environment and ecotoxicology

The soil K_d is 2.09–11.67 mL/g. $K_{oc} = 290–566$. The aerobic soil metabolism DT_{50} is 66–117 days. The oral LD_{50} in mallard duck is >2000 mg/kg, while the dietary LC_{50} is >5300 ppm. The 96-h LC_{50} is >25 mg/L for bluegill sunfish, 3.4 mg/L for rainbow trout, and 14 mg/L for carp. For *Daphnia* the 48-h EC_{50} is 49 mg/L and for algae >20 mg/L.

Appendix: Complete listing of fungicides by chemical classes

[1] ALIPHATIC NITROGEN FUNGICIDES
butylamine
cymoxanil
dodicin
dodine
guazatine
iminoctadine

[2] AMIDE FUNGICIDES
carpropamid
chloraniformethan
cyazofamid
cyflufenamid
diclocymet
ethaboxam
fenoxanil
flumetover
furametrpyr
furametpyr
prochloraz
quinazamid
silthiofam
triforine

[2(a)] **Acylamino Acid Fungicides**
benalaxyl
benalaxyl-M
furalaxyl
metalxyl
metalaxyl-M
perfurazoate

[2(b)] **Benzamide Fungicides**
benzohydroxamic acid
trioxymid
trichlamide
zarilamid
zoxamide

[2(c)] **Furamide Fungicides**
cyclafuramid
furmecyclox

[2(d)] **Phenylsulfamide Fungicides**
dichlofluanid
tolylfluanid

[2(e)] **Vanilamide Fungicides**
benthiavalicarb
iprovalicarb

[2(f)] **Anilide Fungicides**
benalaxyl
benanaxyl-M
boscalid
carboxin
fenhexamid

metalaxyl
metalaxyl-M
ofurace
oxadixyl
oxycarboxin
pyracarbolid
thifluzamide
tiadinil

[2(f)i] *Benzanilide Fungicides*

benodanil
flutolanil
mebenil
mepronil
salicylanilide
tecloftalam

[2(f)ii] *Furanilide Fungicide*

fenfuram
furalaxyl
furcarbanil
methfuroxam

[2(f)iii] *Sulfonanilide Fungicides*

flusulfamide

[3] ANTIBIOTIC FUNGICIDES

aureofungin
blasticidin-s
cycloheximide
griseofulvin
kasugamycin
natamycid
polyoxins
polyoxorims
sreptomycin
validomycin

[3(a)] **Strobin Fungicides**

azoxystrobin
dimoxystrobin
fluoxastrobin
kresoxim-methyl
metominostrobin
orysastrobin
picoxystrobin
pyraclostrobin
trifloxystrobin

[4] AROMATIC FUNGICIDES
biphenyl
chloroneb
chlorothalonil
cresol
dicloran
hexachlorobenzene
pentachlorophenol
quintozene
sodium pentachlorophenoxide
technazine
[5] BENZIMIDAZOLE FUNGICIDES
benomyl
carbendazim
chlorfenazole
cypendazole
debacarb
fuberidazole
mecarbinzid
rabenzazole
thiabendazole
[6] BENZIMIDAZOLE PRECURSOR FUNGICIDES
furophanate
thiophanate
thiophanate-methyl
[7] CARBAMATE FUNGICIDES
benthiavalicarb
furophanate
iprovalicarb
propamocarb
thiophanate
thiphanate-methyl
[7(a)] **Benzimibazolylcarbamate Fungicides**
benomyl
carbendazime
cypendazole
debacarb
mecarbinzid
[7(b)] **Carbanilate Fungicides**
diethofencarb
[8] CONAZOLE FUNGICIDES
[8(a)] **Conazole Fungicides (Imidazoles)**
climbazole

clotrimazole
imazalil
oxpoconazole
prochloraz
triflumizole

[8(b)] **Conazole Fungicides (Triazoles)**

azaconazole
bromuconazole
cyproconazole
diclobutrazol
difenoconazole
diniconazole
diniconazole-M
epoxiconazole
etaconazole
fenbuconazole
luquinconazole
flusilazole
flutriafol
furconazole
urconazole-cis
hexaconazole
imibenconazole
ipconazole
metconazole
myclobutanil
penconazole
propiconazole
prothioconazole
quiconazole
simeconazole
tebuconazole
tertaconazole
triadimefon
triadimenol
triticonazole
uniconazole
uniconazole-P

[9] COPPER FUNGICIDES

Bordeaux mixture
Burgundy mixture
Cheshunt mixture
copper acetate

copper carbonate
copper hydroxide
copper naphthenate
copper oleate
copper oxychloride
copper sulfate
copper zinc chromate
cufraneb
cuprobam
cuprous oxide
mancopper
oxine copper

[10] DICARBOXIMIDE FUNGICIDES

captafol
captan
chlozolinate
dichlozoline
ditalimifos
famoxadone
folpet
iprodione
isovaledione
myclozolin
procymidone
thiochlorfenphin
vinclozolin

[11] DINITROPHENOL FUNGICIDES

binapacryl
dinobuton
dinocap
dinocap-4
dinocap-6
dinocton
dinopenton
dinosulfon
dinterbon
DNOC

[12] DITHIOCARBAMATE FUNGICIDES

azithiram
carbamorph
cufraneb
cuprobam
disulfiram

ferbam
metam
nabam
tecoram
thiram
ziram
[12(a)] **Cyclic Dithiocarbamate Fungicides**
dazomet
etem
milneb
[12(b)] **Polymeric Dithiocarbamate Fungicides**
man copper
mancozeb
maneb
metiram
polycarbamate
propineb
zineb
[13] IMIDAZOLE FUNGICIDES
cyazofamid
fenamidone
fenapanil
glyodin
iprodine
isovaledione
pefurazoate
trazoxide
[14] INORGANIC FUNGICIDES
potassium azide
potassium thiocyanate
sodium azide
sulphur
[15] MERCURY FUNGICIDES
[15(a)] **Inorganic Mercury Fungicides**
mercuric chloride
mercuric oxide
mercurous chloride
[15(b)] **Organomercury Fungicides**
ethyl mercury acetate
ethyl mercury bromide
ethyl mercury chloride
ethyl mercury phosphate

2-methoxyethylmercury chloride
methyl mercury benzoate
methyl mercury dicyandiamide
phenylmercuriurea
phenyl mercury acetate
phenyl mercury chloride
phenyl mercury salicylate
thiomersal
tolylmercury acetate

[16] MORPHOLINE FUNGICIDES
ldimorph
benzamorph
carbamorph
imethomorph
dodemorph
fenpropiomorph
fluomorph
tridemorph

[17] ORGANOPHOSPHATE FUNGICIDES
ampropylfos
ditalimfos
edifenphos
fosetyl
hexythiofos
iprobenfos
phosdiphen
pyrazophos
tolclofos-methyl
triamiphos

[18] ORGANOTIN FUNGICIDES
decafentin
fentin
tributyltinoxide

[19] OXATHIIN FUNGICIDES
carboxin
oxycarboxin

[20] OXAZOLE FUNGICIDES
chlozolinate
dichlozoline
razoxolon
famoxadone
hymexazol

metazoxolon
myclozolin
oxadixyl

[21] PHENYLUREA FUNGICIDE
pencycuron

[22] POLYSULFIDE FUNGICIDES
barium polysulphide
calcium polysulphide
potassium polysulphide
sodium polysulphide

[23] PYRIDINE FUNGICIDES
boscalid
buthiobate
diprithione
fluazinam
pyridinitril
pyrifenox
pyroxychlor
pryroxyfur

[24] PYRIMIDINE FUNGICIDES
bupirimate
cyprodinil
diflumetorim
dimethirimol
ethirimol
fenarimol
ferimzone
mepanipyrim
nuarimol
pyrimethanil
triarimol

[25] PYRROLE FUNGICIDES
fenpiclonil
fludioxonil
fluoroimide

[26] QUINOLINE FUNGICIDES
ethoxyquin
halocrinate
8-hydroxyquinoline sulphate
quinacetol
quinoxyfen

[27] QUINONE FUNGICIDES
benquinox

chloranil
dichlone
dithianon

[28] QUINOXALINE FUNGICIDES
chinomethionat
chlorquinox
thioquinox

[29] THIAZOLE FUNGICIDES
ethaboxam
etridazole
metsulphovax
octhilinone
TCMTB
thiabendazole
thiadifluor
thifluzamide

[30] THIOCARBAMATE FUNGICIDES
methasulphocarb
prothiocarb

[31] THIOPHENE FUNGICIDES
ethaboxam
silthiofam

[32] TRIAZINE FUNGICIDES
anilazine

[33] TRIAZOLE FUNGICIDES
bitertanol
fluotrimazole
tiabutil

[34] UNCLASSIFIED PESTICIDES
acibenzolar
acypetacs
allyl alcohol
bentaluron
benzalkonium chloride
benzamacril
benzohydroxamic acid
bethoxazin
bithionol
carvone
chlobenthiazone
chloropicrin
DBCP
dehydroacetic acid

dichlorophen
diclomezine
diethyl pyrocarbonate
diphenylamine
fenaminosulph
fenitripan
fenpropidin
ferimzone
formaldehyde
hexachlorobutadiene
isoprothiolane
methyl isocyanate
metrafenone
nitrostyrene
nitrothal-isopropyl
OCH
2-phenyl phenol
phthalide
piperalin
probenzaole
proquinazid
pyroquilon
sodium orthophenylphenoxide
spiroxamine
sultropen
thicyofen
tricyclazole
zinc naphthenate

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7 Toxicology of Herbicides*

Timothy C. Marrs

Herbicides

Herbicides are substances that kill plants. They have variable degrees of specificity. Some, for example paraquat, kill all green plants, whereas others, for example the phenoxy compounds, are specific for certain groups of plants. A chemical classification is given in Table 7.1. These compounds, particularly the non-selective examples, are less likely to appear in food than insecticides and fungicides as they are less likely to be used on crops, but exposure of operators can occur as with other pesticides.

Inorganic herbicides

Substances such as common salt (sodium chloride) have been used as herbicides for many years. Indeed, the Romans are reputed to have sterilized the soil of Carthage with salt after the Romans' victory in the third Punic war in 146 BC. The disadvantage with such herbicides, from the agricultural point of view, is that they are non-selective. Nevertheless, sodium chlorate continues to be used as a herbicide and when ingested in man it produces vomiting and abdominal pain, diarrhoea, methaemoglobinaemia, and intravascular haemolysis (Helliwell and Nunn, 1979; Proudfoot, 1996). Sodium chlorate is an oxidizing agent and poses a fire hazard (*Pesticide Manual*, 1991).

Bipyridylium herbicides

This group of pesticides contains two well-known non-selective herbicidal compounds, namely paraquat and diquat (Figure 7.1). In experimental animals and in humans, the mechanism of toxic action of both compounds is very similar at the

*The views expressed in this chapter are those of the author and do not necessarily reflect the views of any UK Government Department or Agency.

Table 7.1 Main groups of herbicides[a]

Group		Examples
Inorganic		Sodium chlorate
Bipyridylium		Parquat Diquat
Organic acid	Phenoxy	2,4-D
		2,4,5-T
		Mecoprop
		Fenoprop
		Haloxyfop
	Other organic acids	Dicamba
Substituted anilines		Alachlor
		Propachlor
		Propanil
Ureas and thioureas		Diuron
		Linuron
		Monolinuron
Nitriles		Ioxynil
		Bromoxynil
Triazines and triazoles	Triazines	Atrazine
		Simazine
		Cyanazine
	Triazoles	Amitrole
Organophosphate group	Phosphonic acid derivatives	Glyphosate
	Phosphinic acid derivatives	Glufosinate

[a]Reproduced from Marrs and Dewhurst (1999), with permission of the authors and Macmillan Reference Ltd.

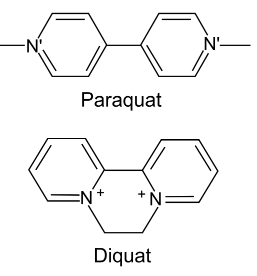


Figure 7.1 Bipyridilium herbicides

molecular level and involves cyclic reduction – oxidation reactions which produce reactive oxygen species and depletion of NADPH. However, the critical target organ differs with the two compounds, so that the mammalian toxicology is quite

different. While both herbicides affect the kidneys, paraquat is selectively taken up in the lungs and the toxicity of paraquat is dominated by lung toxicity. Both can produce local contact toxicity.

Paraquat

Chemical identification

Class: bipyridilium herbicide
Molecular weight: 186.3 (ion), 257.2 (dichloride)
Common name: paraquat
IUPAC name: 1,1-dimethyl-4,4-bipyridinium
CAS name: 1,1-dimethyl-4,4-bipyridinium
Synonyms: methyl viologen
CAS no.: 4685-14-7 (ion) 1910-42-5 (dichloride)

Paraquat is capable of producing both local and systemic toxicity. Local toxicity is produced by direct injury to tissues with which the pesticide comes into contact. Tissues commonly damaged in this way include the skin, the cornea, the larynx, and the mucosa of the upper gastrointestinal tract, the extent and severity of such damage being dependent on the concentration of paraquat in the formulation rather than the dose. Because of the nature of the toxicity of paraquat, this substance is dealt with in more detail than some other herbicides discussed in this chapter.

As discussed above, the systemic toxicity of paraquat is dominated by pulmonary toxicity, which is the result of the active uptake of the compound by the lungs by a saturable uptake process (Rose, Smith, and Wyatt, 1974; Rose *et al.*, 1976; Smith, 1982; Smith *et al.*, 1990). Secondary target organs of toxicity are the kidneys and liver.

Absorption, distribution, and excretion

Dey *et al.* (1990) studied the pharmacokinetics of ^{14}C-paraquat administered to rats as a single sc injection. The dose was such as to produce lung damage but avoid kidney damage. Paraquat was rapidly absorbed with peak blood concentrations at 20 min. The pharmacokinetics were best characterized as a two-compartment open model, the mean $t_{1/2}$ being approximately 40 h. Peak tissue concentrations in the kidney and lung were at 40 min. Hawksworth, Bennett, and Davies (1981) studied the elimination of paraquat in dogs. After intravenous injection of low doses of ^{14}C paraquat, label was rapidly excreted in the urine, the clearance being greater than the glomerular flitration rate, suggesting a process of active secretion. Secretion could be inhibited by N'-nicotinamide. Large doses of paraquat (20 mg/kg bw) produced renal failure as evidenced by a decrease in both renal creatinine and paraquat clearance. The elimination of paraquat could be described by a three-compartment open model.

Mechanism of uptake in the lung

A considerable amount of work has been done on the mechanisms that underly the toxicity of paraquat, and the fact that paraquat is concentrated by the lungs has been discussed above. Rose *et al.* (1976) showed that lung slices from rats, dogs, rabbits, and cynomolgus monkeys could concentrate paraquat actively. Paraquat and the structurally similar polyamines, such as putrescine and spermidine, are accumulated by type II alveolar cells by the polyamine active uptake system (see review by Smith, 1985). Diquat is not a substrate for this system and this fact accounts for the different organ-specific toxicity of the two bipyridilium compounds. Chen, Bowles, and Pond (1992) studied the uptake kinetics of paraquat and putrescine and their mutual inhibition in rat type II alveolar cell suspensions. The uptake of paraquat by type II cells exhibited saturation kinetics and could be inhibited in a concentration-dependent manner by putrescine. The authors postulated that the polyamine uptake pathway in type II cells for paraquat and putrescine possessed two separate sites, one for each substrate, and that binding at one site leads to a conformational change in the other.

Uptake into the brain

A number of investigators have looked specifically at entry of paraquat into the central nervous system, as a result of the suggestion that paraquat may be a factor in the aetiology of Parkinson's disease (see below). Naylor *et al.* (1995) examined the distribution of paraquat in the brain following subcutaneous administration of ^{14}C-labelled paraquat to rats. Following administration, label reached a maximal concentration in the brain (0.05 per cent of the administered dose) within the first hour and then rapidly disappeared from the brain. However, 24 h after administration of the herbicide, about 13 per cent of the maximal recorded concentration of paraquat still remained in the brain and could not be removed by intracardiac perfusion. Most of the paraquat was associated with five structures, two of which (the pineal gland and linings of the cerebral ventricles) lie outside the blood–brain barrier. The remaining three brain areas, the anterior portion of the olfactory bulb, hypothalamus, and area postrema, do not have a blood–brain barrier. The authors concluded that paraquat remaining in the brain 24 h after systemic administration was associated with elements of the cerebral circulatory system, such as the endothelial cells that make up the capillary network, and also that there was limited entry of paraquat into brain regions without a blood–brain barrier. Widdowson *et al.* (1996a) compared the extent of paraquat entry into the brain of neonatal (10-day-old), adult (3-month-old), and elderly (18-month-old) rats. A single dose was administered sc, labelled with ^{14}C-paraquat. The rats were killed 30 min or 24 h after injection, blood taken by cardiac puncture, and the brains removed. Groups of neonatal, adult, or elderly rats were similarly injected and killed 24 or 48 h after dosing, for histopathological examination of the brain. In all three groups, plasma paraquat concentrations were much higher at 30 min than at 24 h. At 30 min the

concentration of paraquat in the brain was highest in the elderly rats, while at 24 h the concentration in the adult and elderly rats' brains had fallen, but it remained high in the brains of the neonatal rats. Autoradiography showed similar distributions of paraquat in the brain regions, paraquat being found in areas outside the blood–brain barrier or where the barrier is incomplete, e.g. dorsal hypothalamus, area postrema, and anterior olfactory bulb. There was no evidence of paraquat-induced cell damage in neonatal brain, although there was increased paraquat entry in that group compared with the older rats. Widdowson *et al*. (1996b) studied the entry of paraquat into the brains of rats. Paraquat labelled with ^{14}C was administered orally, daily for 14 days to five rats while a further five rats received a single oral dose of 5 mg ion/kg bw/day, labelled with ^{14}C. The rats were killed 24 h after the last of the 14 doses or after the single dose. Brain paraquat concentrations were 10 times higher in those rats receiving multiple injections than in those receiving single doses. Shimizu *et al*. (2001) studied rats using a brain microanalysis technique with HPLC/UV detection and found that paraquat (5, 10, or 20 mg/kg bw sc) appeared in the dialysate of the striatum. They also found that paraquat did not facilitate penetration of the blood–brain barrier by 1,2,3,6-tetrahydropyridinium ion. L-Valine injection 30 min before paraquat reduced the striatal extracellular paraquat concentrations. The authors hypothesized that paraquat was taken up into the brain via the neutral amino acid transporter.

Metabolism

Only a small fraction of orally-administered paraquat is metabolized, the greater part being excreted in the urine unchanged. Daniel and Cage (1966) undertook a study in rats using ^{14}C-labelled paraquat dichloride, and some evidence of metabolism was found. Of the oral dose of paraquat, 30 per cent of the label was present in the gut as metabolic products. Furthermore, a small amount of metabolite was present in the urine after oral but not sc administration, suggesting the absorption of metabolites from the gut. Studies *in vitro*, using fecal homogenates, suggested that microbiological metabolism was responsible for this. In a gavage study reported by Murray and Gibson (1974) in rats, guinea pigs, and monkeys, using ^{14}C-labelled paraquat, metabolites were not observed. The metabolism that does occur is via demethylation and oxidation.

Animal toxicology

In experimental animals, the toxicity of paraquat is dominated by effects on the lungs and, to a lesser extent, the kidney. Brooks (1971) carried out studies on small groups of mice exposed to 50–300 ppm in their drinking water and retained for from 1 to 16 weeks. The main findings on light microscopy were vascular dilatation and veins filled with platelets and eryrthocyte aggregates. At the higher doses interalveolar septal thickening was seen. At 100 ppm and above, focal or sometimes lobar pneumonitis

was observed, with small mononuclear cells, macrophages, and neutrophils. In those mice receiving paraquat for 4 weeks or more, fibroblasts were seen in the septal walls. Obliteration of air spaces was seen. The type II cells were observed to be undamaged on electron microscopy in this study, but the type I cells were swollen and there was evidence of oedema of interalveolar septa. The alveolar air spaces were filled with a clear exudate and where there was consolidation, fibroblasts and collagen were observed. Lymphocytes and plasma cells were noted. Subsequent studies have shown damage to other cell types such as the type II alveolar cells and clara cells (see FAO/WHO, 1987). In other species, such as the rat, dog, and monkey, the histopathological appearances are generally similar to those in mice (Clark, McElligott, and Hurst, 1966; Murray and Gibson, 1972), although Butler (1975) found the Syrian hamster relatively resistent to interstitial fibrosis. Butler and Kleinerman (1971) reported that rabbits did not develop the pulmonary changes typical of paraquat poisoning in other species, despite doses of 2–100 mg/kg bw being administered ip and sacrifice of animals being delayed up to 1 month. The only findings in the lungs were occasional small interstitial infiltrates of lymphocytes and plasma cells, minimal alveolar hyperplasia, and some alveolar macrophages. In regulatory studies the changes seen mainly reflect the pneumotoxicity of paraquat. Thus, in short-term studies in rodents and dogs, lung changes occurred: these were also seen in a 1-year dog study. Effects may also be observed in the gastrointestinal tract, the liver, and in the blood.

Long-term toxicity including carcinogenicity

Paraquat is not considered to be carcinogenic; however, in some long-term studies lung tumours have been observed (FAO/WHO, 1987). As with diquat (see below) cataracts have been observed in rats.

Developmental and reproductive toxicity

Neither specific reproductive toxicity nor teratogenicity has been observed except where accompanied by maternal toxicity (see also FAO/WHO, 1987; WHO, 1984). In a study by Bus *et al.* (1975) in mice, no teratogenic effect was observed, although a slight degree of non-ossification of sternabrae was seen at all test doses. Fetotoxicity, as evidenced by increased percent resorption, was seen only at the higher of the two doses used. At no dose was the number of fetuses, or their mean body weight, affected by treatment. Radioactivity reaching the mouse embryo, when ^{14}C-labelled paraquat was administered orally on day 11 of gestation, was low. The developmental toxicity of paraquat was determined in rats by administering paraquat iv at a single dose of 15 mg/kg bw on a single day, one of the days 7–21 of gestation. The number of live and dead fetuses and resorptions was counted at day 22 (or before for decedent dams). Excess maternal deaths occurred with paraquat compared with the saline controls and there was an increase in the number of dead and resorbed fetuses.

Bus and Gibson (1975) administered paraquat in the drinking water at 50 or 100 ppm to mice, exposure starting at day 8 of gestation and continuing until 42 days *post partum*. Neither treatment altered the post-natal growth rate nor did drinking water at 50 ppm increase the post-natal mortality. Drinking water containing paraquat at 100 ppm increased the post-natal mortality, and increased the sensitivity of pups to oxygen toxicity 1 and 28 days after birth, whereas 50 ppm paraquat in the drinking water did not. Both concentrations of paraquat in the drinking water increased the sensitivity to oxygen toxicity and to bromobenzene at 42 days after birth. The authors considered that the effect of bromobenzene might be due to depletion of reduced glutathion.

A two-generation study of the reproductive toxicity of paraquat was undertaken by Dial and Dial (1987). Exposure of the parental (F_0) mice continued until the weaning of the F_1 mice, which were exposed to the diet for 49 days post-natally. No differences were observed in the females' age at first parturition, pups borne/litter, or in pup abnormalities; however, at the highest dietary concentrations the number of pairs of mice producing litters was reduced on account of maternal deaths. Furthermore, the highest dietary concentration produced effects on F_1 offspring mortality. The F_1 females' age at second parturition was increased and the F_2 mortality at 7 weeks was increased. Excess mortality was not observed in the F_1 parents. Maternal and offspring lungs were histopathologically abnormal, with extensive fibrosis.

Production of cell damage

Bus, Aust, and Gibson (1976) studied the hypothesis that the pulmonary toxicity of paraquat is due to cyclic reduction–oxidation, with generation of superoxide radicals and singlet oxygen with the production of lipid peroxidation. Mouse lung microsomes *in vitro* catalysed NADPH-dependent reduction of paraquat. Incubation of paraquat with NADPH, NADPH-cytochrome reductase, and purified microsomal lipid increased malondialdehyde production. Addition of superoxide dismutase or 1,3-diphenylisobenzofuran (a singlet oxygen trapper) inhibited paraquat-induced lipid peroxidation. Paraquat toxicity in mice was decreased by phenobarbital and increased by selenium, vitamin E, or reduced glutathion deficiency. Paraquat toxicity was increased by exposure to 100 per cent oxygen.

Genotoxicity

It is difficult to summarize the data on the genotoxicity of paraquat because of the large number of tests that have been done, the discrepant results, and the non-standard systems used. In many cases the purity of the material was not stated and studies have not been to Good Laboratory Practice (GLP) standards. The majority of Ames tests undertaken on paraquat have been negative (e.g. Benigni *et al.*, 1979; Eisenbeis, Lynch, and Hampel, 1981; Moriya *et al.*, 1983; Nishimura, Nishimura, and Oshima, 1982; Shirasu *et al.*, 1982) or weakly or marginally

positive (Lin, Kuo, and Hsu, 1988; Moody and Hassan, 1982). Of other studies *in vitro* a DNA-repair test in *Salmonella typhimurium* TA 1538 and TA 1978 was positive (Benigni *et al*., 1979). Of the studies *in vivo*, mouse micronucleus tests conducted by Prabakaran and Moorthy (1998), Melchiorri *et al*. (1998) and Ortiz *et al*. (2000) were all positive, whereas that reported by Pena, Mesquita, and Cólus (1999) was negative. Mouse dominant lethal tests reported by Pasi *et al*. (1974) and Anderson, McGregor, and Purchase (1976) were negative.

Effects in humans

Paraquat is a major cause of death from poisoning. Casey and Vale (1994) tabulated deaths from pesticide poisoning from 1945 through 1989 in England and Wales: paraquat was responsible for 570 deaths, which was 56.3 per cent of all deaths caused by pesticides.

Paraquat poisoning usually is the result of ingestion of liquid paraquat formulations available to farmers and professional horticulturists. Much less often, fatal paraquat poisoning may result from ingestion of preparations available for home garden use or from dermal absorption (Garnier *et al*., 1994; Papiris *et al*., 1995). There are numerous case reports and case series of paraquat poisoning (e.g. Bismuth *et al*., 1982; Bramley and Hart, 1983; Bullivant, 1966; Campbell, 1968; Carson and Carson, 1976; Douze *et al*., 1974; Hall, 1995; Malone *et al*., 1971; Naito and Yamashita, 1987; Tsatsakis, Perakis, and Koumantakis, 1996; van Wendel de Joode *et al*., 1996; Wesseling, Castillo, and Elinder, 1993; Wesseling *et al*., 1997).

The effects of paraquat are local and systemic, the former being concentration-dependent, while the latter are dose-dependent (Proudfoot, 1999a). Local effects include damage to the skin, nails, and nose (Bismuth, Hall, and Wong, 1995; Hearn and Keir, 1971; Samman and Johnston, 1969; Vale, Meredith, and Buckley, 1987) and sore throat, dysphagia, and epigastric pain may occur. Local effects to the eye may heal only slowly and with scarring (Devečková, Mráz, and Mydlik, 1980; Peyresblanques, 1969). Ulceration of the upper gastrointestinal tract is often observed. Although the local effects can be severe and unpleasant, it is the systemic effects, largely referable to the respiratory system, that are potentially lethal. Crepitations may be heard and there may be dyspnoea and cyanosis. Radiology initially reveals diffuse fine mottling of the lungs. Renal dysfunction may partly be a direct effect of paraquat and partly be caused by hypovolaemia. Although the degree of renal failure may be mild by most standards, renal failure impairs the only route of excretion available and therefore may contribute significantly to the mortality produced by paraquat. Lung function tests are commonly abnormal (Bismuth *et al*., 1982). The course of the poisoning depends on the amount of paraquat ingested. Ingestion of large amounts (>6 g) of paraquat usually results in death within 36 h, acute pneumonitis, shock, metabolic acidosis, and convulsions commonly being seen. Nausea, vomiting, and abdominal pain are also present. After ingestion of

smaller amounts (3–6 g) death is usually delayed for 5–10 days. Respiratory distress becomes apparent after 4–7 days: radiologically there is opacification of the lungs and hypoxia which becomes increasingly severe as death approaches. The ingestion of amounts of paraquat smaller than 3 g, even as low as 1.5 g may produce death, although the lung effects are likely to be delayed, sometimes considerably so. Initially, there may be nausea, vomiting, and abdominal discomfort together with mild renal impairment. However, dyspnoea may occur after about 10–21 days, death from pulmonary fibrosis occurring up to 6 weeks after exposure. Paraquat concentrations in plasma taken within 24 h of exposure are predictive of the outcome in 90 per cent of cases (Proudfoot, 1995). Proudfoot *et al.* (1979) reported that the plasma paraquat concentration was a good predictor of the outcome in that those whose concentrations were below 2.0, 0.6, 0.3, 0.16, and 0.1 mg/L at 4, 6, 10, 16, and 24 h after ingestion survived. Scherrmann *et al.* (1987) reported that plasma paraquat concentrations in those admitted more than 24 h after poisoning were predictive of the outcome of the poisoning in most patients. Furthermore, they concluded on the basis of 53 patients that those with urinary concentrations of paraquat of less than 1 mg/L within 24 h of exposure would survive, whereas a fatal outcome could be anticipated in most in whom the urinary concentration of paraquat was higher.

The appearence of the lungs at autopsy depends on the survival time. There may be a pleural effusion, and damage to the upper respiratory tract. Grossly, the lungs appear solid, with haemorrhages, including subpleural ones. Histologically there is an initial destructive acute alveolitis, type I alveolar cells being the first cell type affected. Later, type II alveolar cells are affected and clara cells may be destroyed. There follows a proliferative phase, with fibroblastic proliferation in the alveolar walls. Infiltration with mononuclear cells, polymorphs, macrophages, and eosinophils has been reported. The alveoli show oedema and are airless (Marrs and Proudfoot, 2003). The longer the survival time the greater the proliferation of epithelium and fibroblasts in the alveoli (Carson and Carson, 1976). Tubular damage in the kidney has been reported as well as mid-zonal and centrilobular degeneration in the liver.

In a fatal case of paraquat poisoning in a pregnant woman, who developed the typical symptoms and signs of paraquat poisoning and, at *post mortem*, had the typical lung pathology of paraquat poisoning, the fetal lungs were normal (Fennelly, Gallagher, and Carroll, 1968). However, Talbot and Fu (1988), who reported the details of nine pregnant women who ingested paraquat, stated that paraquat in one case was concentrated 4–6 times in the fetus. In another of the cases, the amniotic fluid contained paraquat at twice the concentration in the maternal blood. All the fetuses died, whether or not Caesarian section was carried out.

Although most patients who have radiological lung changes go on to develop progressive and ultimately fatal lung damage, there are a few case reports in which patients have developed persistent radiological changes but have survived (e.g. Hudson *et al.*, 1991). There is also evidence that, in such patients, some recovery

may occur over time (Lin, Liu, and Leu, 1995; Ming, Chun, and Khoo, 1980; Papiris *et al.*, 1995).

The vast majority of paraquat intoxications are by ingestion. However, Athanaselis *et al.* (1983) reported the poisoning of a 64-year-old spray operator via the skin. Fluid had leaked down his back for several hours, causing irritation of the skin. Two days later the sprayman visited a doctor, who advised hospitalization. The patient rejected this advice but was admitted 3 days later into hospital. He died 12 h after this of toxic shock and renal and respiratory insufficiency. At autopsy the findings were typical of paraquat poisoning with fibrosing interstitial pneumonitis and intra-alveolar haemorrhage in the lungs, renal tubular cell degeneration, cholestasis, and necrosis of the skin of the back. A further case of a fatality from transdermal exposure to paraquat was reported from Papua New Guinea, the patient evidently thinking that Gramoxone (20 per cent paraquat w/v), would kill lice, for which purpose he applied the material to his scalp and beard. This produced painful sores and he steadily deteriorated until dying 6 days after applying the paraquat to his skin. At autopsy, there were skin lesions as well as solid and haemorrhagic lungs (Binns, 1976). Garnier *et al.* (1994) reported two cases of percutaneous exposure. In the first case a 36-year-old man applied 20 per cent concentrate to his whole body to cure scabies. He developed extensive erythema followed by blistering and 2 days later he was admitted to hospital. He developed transient renal failure. Dyspnoea appeared one week after admission and he deteriorated, dying 26 days after exposure. The other case reported by Garnier *et al.* (1994) was much milder with mainly skin effects and the outcome was not fatal. Further cases of fatal percutaneous paraquat intoxication were reported by Newhouse, McEvoy, and Rosenthal (1978), Levin *et al.* (1979), Wohlfahrt (1982), Okonek *et al.* (1983), and Papiris *et al.* (1995). In general systemic toxicity after percutaneous exposure of humans seems unusual (Hoffer and Taitelman, 1989). In the case of fatal cases arising from dermal absorption it is likely that the skin was abnormal.

Treatment

There is no specific antidotal treatment for paraquat poisoning, although numerous measures have been tried, many concentrating on the prevention of absorption (Meredith and Vale, 1987). Gastric lavage, fullers' earth, and activated charcoal have all been used. Other therapies that have been investigated include removal of paraquat from the blood by forced diuresis, peritoneal dialysis, haemodialysis, or haemoperfusion using sorbent materials, including charcoal haemoperfusion (Tabei, Asano, and Hosoda, 1982). Corticosteroids have also been tried (Bismuth *et al.*, 1982) as have acetylcysteine and deferoxamine (Lheureux *et al.*, 1995), nitric oxide by inhalation (Eisenman *et al.*, 1998), radiotherapy (Talbot and Barnes, 1988), and lung transplantation. Some measures have at times seemed promising, thus Addo, Ramdial, and Poon-King (1984) reported that treatment with cyclophosphamade, dexamethasone, forced diuresis with frusemide, triamterine, and hydrochlorothiazide

enabled the survival of 15/20 patients. This therapy was combined with routine measures, such as fullers' earth, activated charcoal, and magnesium sulphate to eliminate paraquat from the gut. However, time has shown that none of these measures has achieved consistent success, so that the treatment is symptomatic (see review by Bismuth and Hall, 1995). The aim is to minimize the absorption of paraquat and maximize elimination. Fluid loss should be remedied (Vale, Meredith, and Buckley, 1987). Where pulmonary effects occur, the use of oxygen should be postponed as long as possible since it may exacerbate the pulmonary fibrosis (Bismuth *et al.*, 1982).

Other effects of paraquat in animals and humans

No syndrome of chronic disease has been definitively associated with paraquat. Paraquat is structurally similar to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a drug of abuse that causes a Parkinson's disease-like syndrome. MPTP crosses the blood–brain barrier, and is then metabolized to 1-methyl-4-phenylpyridinium. This compound is transported into dopaminergic neurones, where it acts on mitochondrial electron transport complex 1. As a result, paraquat has been considered as a possible aetiologic factor in Parkinson's disease and, while most animal studies suggest that paraquat does not penetrate the blood–brain barrier well, there is some evidence for transport across the blood–brain barrier by the neutral amino acid transporter (see above).

Experimental studies on the neurotoxicity of paraquat

A number of investigators have studied the effects of paraquat when injected directly into the brain (e.g. Bagetta *et al.*, 1992; Calò *et al.*, 1990; de Gori *et al.*, 1988; Liou *et al.*, 1996). Changes observed have included clinical effects (behavioural abnormalities, seizures), abnormal electrical activity in the brain, and neuropathological changes including necrosis of various cell types. These studies are not easy to interpret as it is very difficult to relate the doses used to those which the central nervous systems of the animals had more conventional modes of administration been used, or to human exposure.

Perry *et al.* (1986) studied the effects of injected MPTP and analogues of MPTP *inter alia* paraquat and reduced paraquat on mice. The dosing schedule for paraquat was three sc injections 3 days apart of 14.5 mg/kg bw each being a maximum tolerated dose, while the schedule for reduced paraquat was six daily doses increasing from 7.3 to 116.3 mg/kg bw, with a total dose of 342 mg/kg bw. This dose was well tolerated. Striatal dopamine was not found to be depleted 1 month after the last injection with paraquat or reduced paraquat, whereas it was severely reduced with MPTP. Brooks *et al.* (1999) investigated the possible role of paraquat in Parkinson's disease, using mice. Paraquat was administered ip to mice and ambulatory behaviour monitored. Substantia nigra dopamine neurone number and striatal

dopamine terminal density were quantified after death. The data indicated that paraquat elicited a dose-dependent decrease in substantia nigra dopaminergic neurones, a decline in striatal dopamine nerve terminal density, and a neurobehavioural syndrome, which was characterized by reduced ambulatory activity. The authors suggested that systemically absorbed paraquat crossed the blood–brain barrier to cause destruction of dopaminergic neurons in the substantia nigra and reduction of dopaminergic innervation of the striatum.

Effects of paraquat by the oral route

In the study by Widdowson *et al.* (1996b) on the entry of paraquat into the brains of rats, discussed above, groups of rats were dosed daily for 14 days with water (controls) or 5 mg/kg bw paraquat ion orally. The brains were processed for histopathological examination. There was no sign of neuronal cell damage in the test group, in particular in the substantia nigra. Neurotoxic effects following neonatal exposure to paraquat and MPTP were studied by Fredriksson, Fredriksson, and Eriksson (1993). Five groups of mice were given vehicle (water), paraquat, or MPTP by mouth, the doses of MPTP being 0.3 and 20 mg/kg bw and of paraquat 0.07 and 0.36 mg/kg bw, when 10 and 11 days old. Neonatal spontaneous motor activity was tested on day 18 in mice given paraquat 0.36 mg/kg bw. Adult spontaneous motor activity was tested at ages 60 and 120 days. On day 125 the mice were decapitated and the contents of dopamine and serotonin and metabolites in the striatum were analysed. Acute toxicity was not observed in any of the groups. No respiratory distress or motor performance dysfunction was seen on day 18 in mice given paraquat 0.36 mg/kg bw. The behavioural tests at 60 days of age showed a marked hypoactive condition in the mice given paraquat (at both doses) and MPTP (at both doses). At the age of 120 days the hypoactivity persisted and appeared even more pronounced. Reduced striatal content of dopamine and metabolites was seen in the striatum with both compounds, but serotonin levels were unaffected. The effect was greater at the higher doses. In a study in two strains of mice, one (C57 black) being the same as that used by Fredriksson, Fredriksson, and Eriksson (1993), paraquat was administered as single daily doses of 0.36 or 3.6 mg/kg bw to pups at 10 and 11 days after birth together with appropriate controls (Ray, personal communication, 2003). Spontaneous behaviour testing was carried out at 4 months, and approximately 1 week later the mice were killed and analysed for neurotransmitters in the brain, as well as muscarinic receptor density. In the C57 black mice at 4 months, there was hyperactivity in the 0.36 mg/kg bw group compared with the controls, while at 3.6 mg/kg bw and in the other strain of mice used (NMRI) at both doses there were no significant differences from the controls. There were no significant intergroup differences in muscarinic receptor density nor in striatum or hippocampus dopamine, metabolites of dopamine, or 5-hydroxyindoleacetic acid. The authors concluded that under similar conditions to those used in the Fredriksson study, they could not replicate it.

The significance of these findings for the risk assessment of paraquat in humans remains very unclear.

Interaction of paraquat with other pesticides

Thiruchelvam *et al.* (2000a, 2000b) carried out studies to assess the potential involvement of combined exposure to the herbicide paraquat and the manganese-containing ethylene*bis*dithiocarbamate fungicide, maneb, in the aetiology of idiopathic Parkinson's disease.

Thiruchelvam *et al.* (2000a) evaluated the effects of paraquat dichloride (5 or 10 mg/kg bw) and/or maneb (15 or 30 mg/kg bw) on mice, when given once weekly for a total of 4 weeks, by ip injection. Endpoints assessed were effects on locomotor activity, density of tyrosine hydroxylase positive neurons, levels of dopamine and metabolites, and dopamine turnover. The authors noted that decreases in motor activity immediately following injections were observed more consistently with combined exposures to maneb/paraquat. Levels of dopamine and metabolites and dopamine turnover were slightly increased immediately post-injection by combined exposures compared with exposure to maneb alone. In addition, significant reductions in tyrosine hydroxylase immunoreactivity, measured 3 days after the last injection, were detected in the dorsal striatum of animals given combined treatments, but not those treated with single compounds. The authors concluded that these results demonstrated potentiating effects on nigrostriatal dopamine systems of combined exposures to paraquat and maneb. Thiruchelvam *et al.* (2000b) described similar experiments in mice, treated with single compounds (10 mg/kg bw paraquat, 30 mg/kg maneb) or a combination (10 mg/kg bw paraquat + 30 mg/kg bw maneb), twice weekly by ip injection for 6 weeks. It was reported that maneb, but not paraquat, reduced motor activity immediately after treatment, and that this effect was potentiated by combined paraquat/maneb treatment. As treatments progressed, only the combined paraquat/maneb group showed a failure of motor activity levels to recover within 24 h. Paraquat/maneb in combination, but neither singly, reduced tyrosine hydroxylase and dopamine transporter immunoreactivity in the dorsal striatum, but not the nucleus accumbens. Reactive gliosis occurred only in response to combined paraquat/maneb in the dorsal-medial but not the ventral striatum. Tyrosine hydroxylase immunoreactivity and cell counts were significantly reduced only by the mixture of paraquat and maneb and not by the pesticides alone in the substantia nigra, while no treatment produced significant effects on tyrosine hydroxylase immunoreactivity and cell counts in the ventral tegmental area. The authors suggested that the combination of paraquat/maneb showed synergistic effects, preferentially expressed in the nigrostriatal dopamine system, suggesting that such mixtures could play a role in the aetiology of Parkinson's disease. However, the study design was such that the results could have reflected types of combined action other than synergy.

Epidemiological studies

Rajput *et al.* (1987), who analysed all early age onset Parkinson's disease cases born and raised in Saskatchewan, found that 20 of 22 were exclusively exposed to a rural environment during the first 15 years of life. This distribution was significantly different from the general population. Included in the study was a review of pesticide usage from Saskatchewan agricultural records to determine if there was an increased incidence of early onset Parkinson's disease following utilization of any particular chemical. No increase was found in the incidence of the disease with the introduction of any pesticide, including paraquat, for agricultural use. In a case–control study, Hertzman *et al.* (1990) compared personal histories of 57 cases and 122 age-matched controls to identify possible determinants of Parkinson's disease. Odds ratios adjusted for sex, age, and smoking were computed using stepwise logistic regression. A statistically significant increased risk for working in orchards was found. The relative risk of Parkinson's disease decreased with smoking, an inverse relationship supported by many studies. A questionnaire-based case–control study to investigate possible risk factors for Parkinsonism was undertaken by Koller *et al.* (1990). There were 150 patients with Parkinson's disease and 150 age- and sex-matched controls. Well water use and rural living were associated with Parkinsonism, but farming and pesticide/herbicide use was not. Semchuk, Love, and Lee (1991) undertook a case–control study of 130 cases of Parkinson's disease and 260 age- and sex-matched controls from Calgary, Alberta. No significant association with rural or farm living or drinking well water in early childhood and Parkinson's disease was found. Hertzman *et al.* (1994) carried out a retrospective case–control study, with 127 cases and 245 controls to identify possible risk factors for idiopathic Parkinsonism. Of the controls, 121 had cardiac disease and 124 were randomly selected from electoral lists. An occupational history was collected, as was known contact with all pesticides associated with the tree fruit sector of the agricultural industry. There was a significant association between Parkinsonism and having had an occupation in which exposure through handling or directly contacting pesticides was probable, but no specific chemical was associated with the condition. The authors concluded that although occupations involving the use of agricultural chemicals might predispose to the development of Parkinsonism, it was likely that the pathogenesis is multifactorial rather than related to a specific agent.

A cross-sectional study was undertaken by Castro-Gutiérrez *et al.* (1997) in Nicaragua in order to evaluate any relationship between respiratory health and paraquat exposure. The study population was selected from among workers at 15 banana plantations that used paraquat as a herbicide. All workers who reported never having applied paraquat and all who reported more than 2 years of cumulative exposure as sprayers of paraquat with knapsack sprayers were invited for medical examination. There were 134 workers in the paraquat exposed group and 152 unexposed workers. All took part in a questionnaire interview asking about exposure and respiratory symptoms, and they underwent spirometric testing of forced expiratory volume in 1 s ($FEV_{1.0}$) and forced vital capacity (FVC). Of the exposed group 53 per cent reported having experienced a skin rash or burn resulting from paraquat exposure,

25 per cent reported epistaxis, 58 per cent nail damage, and 42 per cent paraquat splashes of the eyes. There was a consistent relationship between a history of skin rashes or burns and the prevalence of dyspnoea. This relationship was more marked for more severe dyspnoea. There was a 3-fold increase in episodic wheezing accompanied by shortness of breath among the more intensely exposed workers. There was no relationship between exposure and $FEV_{1.0}$ or FVC. The authors considered that the high prevalence of respiratory symptoms associated with exposure, in the absence of spirometric abnormalities associated with exposure, could be a result of unmeasured gas exchange abnormalities among workers with long-term exposure to paraquat. They could also have been caused by recall bias.

Studies in human volunteers

A study *in vivo* of the percutaneous absorption of paraquat was undertaken in six human volunteers by Wester *et al.* (1984). It was concluded that paraquat was poorly absorbed through human skin and that there was little difference between skin at different sites in ability to absorb paraquat.

Reference values

The Joint Expert Meeting on Pesticide Residues (JMPR) ADI is 0.004 mg/kg bw (as paraquat ion), based upon the NOAEL from a dog study, based upon histopathological changes in the lungs and a 100-fold safety factor (FAO/WHO, 1987).

Diquat

Chemical identification

Class: bipyridilium
Molecular weight: 184.2 (ion), 344 (dibromide)
Common name: diquat
IUPAC name: 1,1′-ethylene-2,2′-bipyridyldiylium
CAS name: 6,7-dihydropyrido[1,2-*a*:2,1-*c*]pyrazinediium
CAS no.: 2764-72-9 (ion) 85-00-7 (dibromide)

Diquat is not actively taken up by the lungs and lung changes are not usually found in either animal studies or human exposures.

Absorption, distribution, metabolism, and excretion

Orally-administered diquat is poorly absorbed from the gastrointestinal tract of rats, cows, and goats and mainly eliminated via the faeces, the small fraction absorbed being principally eliminated via the urine. Diquat is not metabolized to a great extent and most is eliminated unchanged.

Animal toxicity

In a 90-day feeding study in rats, reductions in body weight gain and food consumption together with reduced plasma protein were the main effects observed, while in a 1-year feeding study in dogs lens opacities were seen. In long-term/carcinogenicity studies in mice and rats, the effects observed have been rather non-specific, such as reduced growth rates and changes in organ weights. Cataracts were observed in long-term studies in the rat (FAO/WHO, 1994).

Numerous teratology studies have been carried out and diquat does not appear to have teratogenic potential in the species studied. Moreover, there is no indication that diquat has a propensity to produce specific reproductive toxicity.

Genotoxicity

In a range of studies *in vitro* and *in vivo*, no mutagenic response was observed. A response was seen in two cytogenetics assays but only at cytotoxic doses (FAO/WHO, 1994).

Effects in humans

As with paraquat, both local and systemic effects can occur, the former including damage to the oropharynx after ingestion of diquat, while skin contact can produce skin burns (Manoguerra, 1990). Ulceration of the gastrointestinal mucosa, paralytic ileus, and hypovolemic shock may occur. Systemically, renal effects are prominent and in humans, death from ingestion of large amounts is from renal failure (Vanholder, Colardyn, and de Rueck, 1981) (See the review by Jones and Vale, 2000).

Treatment

Initial treatment comprises replacment of fluids and electrolytes lost. Gut decontamination may be considered in the event that life-threatening amounts of diquat have been ingested (Jones and Vale, 2000).

Reference doses

The 1993 JMPR assigned an ADI of 0–0.002 mg/kg bw: this was based upon a 2-year rat study, in which cataractogensis was observed (FAO/WHO, 1994).

Phenoxy acid herbicides

The phenoxy herbicides are chemical analogues of auxins, a type of plant growth hormone and these herbicides produce uncontrolled and lethal growth in target

Figure 7.2 Some phenoxy herbicides

plants. There is no analogous system in animals and the phenoxy herbicides do not act as hormones in animals. Because the phenoxy herbicides are very effective selective weed-killers they are widely used. The most important members of the groups are 2,4-D, MCPA, mecoprop, and dichlorprop (Figure 7.2) and these are the ones most likely to be encountered in acute human poisoning. 2,4,5-T and fenoprop are other members of the group. 2,4,5-T is not used in the United Kingdom and has largely been withdrawn from use elsewhere because of concerns that arose from contamination of some formulations with dioxins (see below). Commercial formulations of phenoxy herbicides commonly contain two or more members of the group, sometimes in combination with ioxynil or dicamba. The chlorophenoxy herbicides are largely excreted unchanged in the urine and have long plasma half-lives (in the range 95–220 h for 2,4-D, MCPA, and dichlorprop) in humans. The acute toxicity of the phenoxy herbicides tends to be moderate to low.

Phenoxy herbicides and malignancy

In humans, the phenoxy herbicides have attracted some suspicion because of epidemiological studies showing positive associations with the manufacture or application of phenoxy herbicides with non-Hodgkin's lymphoma or with soft tissue sarcoma.

Numerous case–control studies have been carried out on occupationally exposed groups and have sought a connection between farming or other occupations and non-Hodgkin's lymphoma. Some case–control studies have looked specifically at exposure to phenoxy herbicides (e.g. Cantor *et al.*, 1992; Hardell and Eriksson,

1999; Hardell *et al.*, 1981; Pearce, Smith, and Fisher, 1985; Persson *et al.*, 1989; Weisenburger, 1990; Woods, Polissar, and Severson, 1987; Zahm *et al.*, 1990). Some studies have found mildly elevated odds ratios when relating exposure to phenoxy herbicides and non-Hodgkin's lymphoma occurrence. Thus, Hoar *et al.* (1986) found an odds ratio of 2.2 (95 per cent confidence limits 1.2–4.1) for those who had ever used phenoxy herbicides, and Hardell and Eriksson (1999) found an odds ratio of 1.5 (95 per cent confidence limits 0.9–2.4) for exposure to phenoxy herbicides. However, a number of the other studies found no such elevation. Moreover, there are some difficulties with interpreting those studies, particularly in quantifying exposure, and there are difficulties with the design of the studies, such as the possibility of recall bias.

Cohort studies generally supply stronger evidence than case–control studies, because recall bias is less of a problem. Cohort studies on chemical workers, e.g. those by Ott, Holder, and Olson (1980), Bishop and Jones (1981), Lynge (1985), Coggon *et al.* (1986), Bond *et al.* (1988), Coggon, Pannett, and Winter (1991), Becher *et al.* (1996), and Burns, Beard, and Cartmill (2001) have recorded the occurrence of non-Hodgkin's lymphoma in a population potentially exposed to phenoxy herbicides. In the Burns *et al.* (2001) cohort the standardized mortality ratio was 1 (95 per cent condidence limits 0.21–2.92), in comparison with rates for non-Hodgkin's lymphoma in the United States. A study of a large international cohort of production workers and sprayers (18 910) exposed to phenoxy herbicides and chlorophenols was reported by Johnson, Winkelmann, and l'Abbé (1990) and Saracci, Kogevinas, and Bertazzi (1991). This cohort included those of Lynge (1985), Coggon, Pannett, and Winter (1991), and Green (1991). No excess of non-Hodgkin's lymphoma was observed overall in the exposed population (11 observed, 11.64 expected, standardized mortatility ratio 0.95, 95 per cent condidence limits 0.47–1.69). For production workers alone, 8 cases of non-Hodgkin's lymphoma were seen as against 5.36 expected (standardized mortatility ratio 1.49, 95 per cent confidence limits 0.64–2.94), whereas for sprayers, there were fewer cases of non-Hodgkin's lymphoma than expected. Of those cohort studies that have been undertaken on agricultural or forestry workers, those by Riihimäki, Asp, and Hernberg (1982), Wiklund, Dich, and Holm (1987), Wiklund, Lindefors, and Holm (1988), Wigle *et al.* (1990), Green (1991), and Asp *et al.* (1994) are noteworthy; these all gave relative risks of <1 for non-Hodgkin's lymphoma or found no cases of the disease. Fleming *et al.* (1999), in a study of pesticide applicators, found a non-significant increased standardized mortality ratio in females only. A retrospective cohort study on lawn-care workers reported a standardized mortatility ratio of 1.14 (95 per cent confidence intervals 0.31–2.91) for non-Hodgkin's lymphoma (Zahm, 1997). A particularly large study was undertaken by Wiklund, Lindefors, and Holm (1988). The exposed population was 350 000 male Swedes employed in agriculture or forestry between 1961 and 1979. These individuals were thought to have been exposed to various phenoxy herbicides, mainly MCPA, and also 2,4-D and 2,4,5-T and comparison was with 1 700 000 men in other employment. There

were 861 cases of non-Hodgkin's lymphoma in the study cohort and 3500 in the reference cohort, giving a relative risk of 1.2 (confidence intervals not given). The study cohort was sub-divided into sub-cohorts by more precise employment criteria (animal husbandry, horticulture, other agricultural, forestry, timber workers, etc.). Few relative risks greater than 1 were seen and no significantly elevated risk. There is therefore little evidence from cohort studies that there is a causal relationship between occupational exposure to phenoxy herbicides and non-Hodgkin's lymphoma. However, the results of most studies are diluted by an absence of quantitative knowledge of exposure and there were also problems with multiple exposure, particularly in those studies looking at farmers and farm workers, although some studies have attempted to allow for exposure to different chemicals, including pesticides. A further difficulty in interpretation is the possible contamination of the pesticides used with TCDD and other substances.

Some other epidemiological studies have linked non-Hodgkin's lymphoma with phenoxy herbicides. Thus, Vineis *et al.* (1991) found higher incidence rates of non-Hodgkin's lymphoma in areas with high levels of phenoxy herbicides in soil or water.

A number of case–control studies on soft tissue sarcoma, in relation to phenoxy herbicides and/or chlorophenols, have been carried out and positive associations have been found in some (e.g. Hardell *et al.*, 1981; Hardell and Sandström, 1979; Vineis *et al.*, 1987). In others, no association was found (Hoar *et al.*, 1986; Smith *et al.*, 1984; Woods, Polissar, and Severson, 1987). As with the epidemiological studies on non-Hodgkin's lymphoma and these compounds, discussed above, there were problems with quantifying exposure, confounding variables, and other aspects of study design. Again, the possibility of recall bias is a major problem.

A number of cohort studies have been undertaken. There was an increased occurrence of soft tissue sarcomas in the Danish study on workers in pesticide manufacturing (relative risk 2.7, 95 per cent confidence intervals 0.88–6.3) (Lynge, 1985). Coggon, Pannett, and Winter (1991), Fleming *et al.* (1999), Asp *et al.* (1994), and Burns, Beard, and Cartmill (2001) indentified no case of soft tissue sarcoma in their cohorts. In the study of Riihimäki, Asp, and Hernberg (1982) there was no excess of soft tissue sarcoma. In the Saracci study, previously discussed, of pesticide production workers and sprayers, the standardized mortality rate for soft tissue sarcoma was 1.96 (95 per cent confidence intervals 0.53–5.02) in the whole cohort but 2.97 (95 per cent confidence intervals 0.61–8.68) specifically in the sprayers.

A number of studies on ex-military personnel who served in the Vietnam war have been carried out (Breslin, Kang, and Lee, 1988; Dalager *et al.*, 1991; O'Brien, Decouflé, and Boyle, 1991). These studies were reviewed by Boyle, Decouflé, and O'Brien (1989). The particular interest in relation to phenoxy herbicides is that the soldiers were exposed to pesticides of the phenoxy group, as well as the insecticide malathion. Of greatest interest was agent orange, a 50/50 mixture of 2,4-D and 2,4,5-T containing small amounts of TCDD. However, exposure of servicemen on active duty appeared to be small as, despite a long $t_{1/2}$ in fat, dioxin levels were no

higher in these men than in soldiers who had not served in Vietnam. It has to be concluded that the evidence linking service in Vietnam and exposure to phenoxy herbicides was equivocal. One further problem with all these studies is that major differences between Vietnam veterans and control populations (variously military personnel who did not serve in Vietnam and the general population of the United States) have been identified in respect of drug-taking and psychological stress: there were also differences in racial and socioeconomic composition between those who served in Vietnam and those who did not. An Australian Royal Commission (1985) concluded, on the basis of some animal studies and data on Vietnam veterans, that cancer had not been induced in Australian personel who had served in Vietnam.

2,4-D

Chemical identification

Class: phenoxy acid
Structural formula: see Figure 7.2
Molecular weight: 221
Common name: 2,4-D
IUPAC name: (2,4-dichlorphenoxy)acetic acid
CAS name: (2,4-dichlorphenoxy)acetic acid
Cas no.: 94-75-7

2,4-D may be taken as the type compound in this group. It has been extensively studied in experimental animals and numerous human exposures have been reported. 2,4-D may be present in formulations as a salt of one or more amines or in the form of an ester. The toxicity of all these forms of 2,4-D is very similar since hydrolysis of the esters and dissociation of the salts takes place very rapidly *in vivo*.

Absorption, distribution, metabolism, and excretion

In experimental animals, absorption and excretion were reported to be rapid, but excretion is saturable at doses above 50 mg/kg bw, which may explain the prolonged half-life in cases of overdose in humans. In animals, no metabolites other than conjugates have been reported (FAO/WHO, 1997). Some human volunteer data are available on absorption, distribution, and excretion of 2,4-D.

Animal toxicology

2,4-D, its salts and esters have acute oral LD_{50}s ranging from 400 mg/kg bw to 2 g/kg bw. Large doses (>175 mg/kg bw) in dogs produced hypotonia (Beasley *et al.*, 1991). This herbicide is not irritant, nor does it have sensitization potential. In both subchronic and long-term studies the primary target organ for toxicity is the

kidney, although in some studies histopathological changes have been seen in the adrenals (hypertrophy of the zona glomerulosa) and liver (hepatocellular hypertrophy and/or necrosis). Furthermore, in rats follicular cell hypertrophy of the thyroid gland, together with decreased thyroxine levels, have been reported. In the kidneys, vacuolization has been observed, together with loss of the brush border and other changes in the cells of the renal proximal tubules. In some studies these changes have been accompanied by rises in blood urea and creatinine. In long-term animals studies there is no evidence of tumourigenic potential. 2,4-D is neither a reproductive toxin nor, with the possible exception of the triisopropanolamine salt, is there evidence of developmental toxicity. The JMPR concluded that 2,4-D was not genotoxic (FAO/WHO, 1997).

Effects in humans

Some human volunteer studies were considered by the JMPR (FAO/WHO, 1997). These showed that, at low doses, 2,4-D was rapidly absorbed and excreted and that transdermal absorption was poor.

Acute poisoning

In humans, a number of cases of acute poisoning have been reported, in which large amounts of 2,4-D have been ingested. The results may be life-threatening (Berthelot-Moritz *et al.*, 1997). As is discussed above, the effects may be prolonged because of the long plasma half-life of 2,4-D at high doses. Poisoning is characterized by vomiting, abdominal pain, hypotension, muscle hypotonia, and/or fasciculation and depression of consciousness. Shock, convulsions, and coma may occur and the last is often prolonged. Effects that may be due to contact, such as burning sensations in the mouth, are sometimes seen (Jorens *et al.*, 1995; Stevens and Sumner, 1991). Where death occurs, in some cases it is due to cardiogenic shock (Jorens *et al.*, 1995).

As there is no specific antidote for 2,4-D poisoning, supportive measures have to be used. Alkalinization of the urine is reported to increase the elimination of 2,4-D in the urine, and thus reduce the plasma half-life to 3–8 h (Flanagan *et al.*, 1990; Proudfoot, 1999b). Intravenous infusion of sufficient alkali (sodium bicarbonate) to induce urine pH values in the region of 8 produces rapid clinical improvement.

Reference dose

The JMPR set an ADI for 2,4-D (as the sum of its salts and esters) of 0.01 mg/kg bw (FAO/WHO, 1997). This is based upon a 1-year dog study and a 2-year rat study, both with NOAELs of 1 mg/kg bw/day. In the dogs at the next highest dose, there were histopathological changes in the livers and kidneys and increases in blood urea and creatinine. In the rats, histopathological changes in the renal tubular cells were observed at the next highest dose.

2,4,5-T

Chemical identification

Class: phenoxy acid
Structural formula: see Figure 7.3
Molecular weight: 255
Common name: 2,4,5-T
IUPAC name: (2,4,5-trichlorphenoxy)acetic acid
CAS name: (2,4,5-trichlorphenoxy)acetic acid
CAS no.: 93-76-5

Of the other important members of the group, 2,4,5-T is no longer much used in developed countries. In laboratory animals, 2,4,5-T is similar in toxicity to 2,4-D but spasticity has been reported in dogs (Drill and Hiratzka, 1953). There is evidence that 2,4,5-T is teratogenic in mice and rats (Courtney *et al*., 1970; Gaines *et al*., 1975; Roll, 1971) but not rabbits, monkeys, or sheep (FAO/WHO, 1996). These studies have to be interpreted in the light of the content of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) of the preparation of 2,4,5-T used, but some studies that have used 2,4,5-T with no detectable TCDD contamination have shown teratogenicity (see Stevens and Sumner, 1991, for a review).

Other phenoxy herbicides

The target organs for the other phenoxy herbicides seem similar to those of 2,4-D. Thus, in experimental animals, fenoprop causes changes in the liver and kidneys (USEPA, 1988).

Human poisonings

Reports of human poisonings with phenoxy herbicides other than 2,4-D are not frequent. In two cases of mecoprop poisoning, unconsciousness, inadequate respiration, hypotension, muscle cramp, and rhabdomyolysis were seen. Renal failure

HO
Cl
O
O
Cl
Cl

Figure 7.3 2,4,5-T

Figure 7.4 Dicamba

occurred (Meulenbelt *et al.*, 1988). A case of MCPA poisoning was reported by Schmoldt, Iwersen, and Schlüter (1997). The patient suffered burning of the mouth, pain in the extremities, and hypotension. Alkalinization of the urine is reportedly effective in the management of poisoning with MCPA and dichlorprop but its effect on mecoprop elimination is less impressive (Proudfoot, 1999b; Schmoldt, Iwersen, and Schlüter, 1997).

Other organic acid herbicides

Dicamba (Figure 7.4) is a herbicide of low acute toxicity. It causes muscular spasms, hypotonia, and dyspnoea in acute studies in animals, whilst in longer-term studies the effects observed are non-specific (Beasley *et al.*, 1991; Stevens and Sumner, 1991). Dicamba is reported to cause peroxisome proliferation (Espandiari, Ludewig, and Robertson, 1998). The effects of acute poisoning with dicamba in humans are not known; it is generally ingested in conjunction with chlorophenoxy herbicides whose actions predominate (see above).

Fluazifop, fluazifop-P, and haloxyfop are selective weedkillers that interfere with plant growth; they are phenoxypropionic acid derivatives. In mice haloxyfop is notable for producing liver tumours in association with peroxisome proliferation (FAO/WHO, 1996). Haloxyfop has a JMPR ADI of 0.0003 mg/kg bw. This is based upon the NOAEL from a 2-year mouse study, in which histopathological changes were observed at the next highest dose, in the liver, including hepatic foci. Liver tumours were observed at higher doses.

Substituted anilines

Alachlor, propanil, metolachlor, and propachlor are aniline derivatives (Figure 7.5). Alachlor, propachlor, and metolachlor are tertiary amines, whereas propanil is a secondary amine. Reduced haemoglobin and reticulocytosis has been observed in rats (WHO, 1993) with propachlor. In repeated-dose animal studies the main target organs are the liver and kidneys (WHO, 1993). Alachlor is, like other substituted aniline herbicides, not a substance of high acute toxicity (*Pesticide Manual*, 1994).

Alachlor

Propanil

Metolachlor

Propachlor

Figure 7.5 Substituted aniline herbicides

It is carcinogenic in rodents, producing posterior nasal, thyroid, and stomach tumours, probably by non-genotoxic mechanisms; it has been opined that these tumours are not likely to be predictive of such effects in humans (Berry, 1988; Heydens *et al.*, 1999; MAFF, 1986).

Effects in humans

Relatively few acute poisonings have been reported in humans with these compounds. With propanil poisoning in humans, methaemoglobinaemia has been reported, severe enough to require treatment with methylene blue (de Silva and Bodinayake, 1997). In contrast, with propachlor, there have been few reports of symptomatic human exposure: the few adverse effects reported with propachlor have been cutaneous (WHO, 1993).

Ureas and thioureas

The herbicidal ureas such as diuron, linuron, and monolinuron (Figure 7.6) appear to interfere with photosynthesis in plants and are of low acute mammalian toxicity. Linuron is a weak androgen receptor antagonist (Lambright *et al.*, 2000). In man, large amounts the herbicidal ureas cause methaemoglobinaemia, intravascular haemolysis, and haemoglobinuria (Casey, Buckley, and Vale, 1994; Proudfoot, 1996).

Diuron

Linuron

Monolinuron

Figure 7.6 Herbicidal ureas

Ioxynil

Bromoxynil

Figure 7.7 Nitrile herbicides

Nitriles

The nitrile herbicides, ioxynil and bromoxynil (Figure 7.7), may uncouple oxidative phosphorylation and/or inhibit oxidative phosphorylation (Stevens and Sumner, 1991). Ioxynil, presumably due to its iodine content, causes enlargement of the thyroid gland in the rat (MAFF, 1986).

Triazines and triazoles

Atrazine, cyanazine, and simazine (triazines) (Figure 7.8) and amitrole (a closely-related triazole) are broad spectrum, widely used herbicides that inhibit photosynthesis. They are of low toxicity. Atrazine, simazine, and cyanazine produce mammary tumors in Sprague-Dawley rats (Bogdanffy *et al.*, 2000; Eldridge *et al.*, 1999), which may be related to the observation that atrazine disrupts hypothalamic control

Atrazine

Cyanazine

Simazine

Figure 7.8 Triazine herbicides

of prolactin and LH production (Cooper *et al.*, 2000). The weight of evidence is that these compounds are non-genotoxic (Kligerman *et al.*, 2000). In rodents, dogs, and sheep, amitrole causes hyperplastic changes, including tumours, in the thyroid (FAO/WHO, 1998; Steinhoff *et al.*, 1983) together with reductions in T3 and T4 (Mattioli *et al.*, 1994). Amitrole is also a catalase inhibitor (Lopez-Torres, Perez-Campo, and Barja de Quiroge, 1990).

Effects in humans

Human poisonings with this group of herbicides have not been common and the effects of ingestion by humans of these compounds tends to be non-specific. In a fatal combined poisoning with amitrole and ammonium thiocyanate, severe oliguria and metabolic acidosis was reported (Legras *et al.*, 1996). Inhalation of an amitrole-containing aerosol is alleged to have been the cause of cough, pulmonary crackles, bilateral pulmonary infiltrates, bilateral pleural effusions, and lung function test results consistent with a restrictive abnormality (Balkisson, Murray, and Hoffstein, 1992).

Reference doses

Amitrole has a JMPR ADI of 0.002 mg/kg bw (FAO/WHO, 1998). This is based upon the NOAELs from a 90-day dietary study and a two-generation study of reproductive toxicity, both in the rat. In the former the goitre was observed. In the latter case effects

observed at the LOAEL were decreased mating and fertility in both sexes and decreased litter size, pup survival, and pup body weight. Additionally, thyroid weight was increased, and histopathologically, follicular hyperplasia was seen. The JMPR considered that rats were more sensitive to the goitrogenic effects of chemicals than were humans and the ADI was calculated using a 50-fold safety factor.

Organic phosphorus herbicides

Two organophosphorus compounds, glyphosate and glufosinate, have low or nonexistent anticholinesterase effects and are used as herbicides. Genetically modified crops with resistance to glyphosate and glufosinate ammonium are being developed.

Glyphosate

Chemical identification

Class: phosphonate
Structural formula: see Figure 7.9
Molecular weight: 169
Common name: glyphosate
IUPAC name: *N*-(phosphonomethyl)glycine
CAS name: *N*-(phosphonomethyl)glycine
CAS no.: 1071-83-6

Glyphosate (*N*-phosphonomethyl glycine) inhibits the enzyme enolpyruvylshikimate phosphate synthase in plants, which is an essential enzyme in the biosynthesis of phenylalanine, tyrosine, and tryptophan. This pathway is not present in animals.

Animal studies

Glyphosate is of very low acute toxicity in experimental animals, the LD_{50} being approximately 5 g/kg body weight (Atkinson, 1985; FAO/WHO, 1987). Products contains either isopropylammonium, sesquisodium, or trimethylsulphonium

Figure 7.9 Glyphosate

together with a surfactant that may be the main cause of toxicity of the early formulations. Glyphosate is poorly absorbed from the gastrointestinal tract and excreted unchanged in the urine. It has been reported that glyphosate, at high concentrations *in vitro* (IC_{50} about 700 mM) can inhibit serum acetylcholinesterase (El-Demerdash, Yousef, and Elagamy, 2001), however there is no indication that significant cholinesterase inhibition occurs in mammals *in vivo*. In repeated-dose studies in experimental animals the toxicity tends to be rather non-specific, failure to gain weight being the most frequent observation. Since very high dietary concentrations were used in some of these studies, this effect may be due to unpalatability of the diet and caloric dilution. There is no evidence of carcinogenic potential in long-term studies nor of teratogenic potential. There is little evidence of genotoxicity in a variety of *in vitro* and *in vitro* tests (Williams, Kroes, and Munro, 2000).

Acute poisoning in humans

High doses are necessary to cause death in humans (Talbot, Shiaw, and Huang, 1991). The common features of poisoning include burning sensations in the mouth and throat accompanied by nausea, vomiting, dysphagia, and diarrhoea. Less frequently, the larynx may be contaminated leading to dysphonia and difficulty in coughing (Hung, Deng, and Wu, 1997; Lee *et al*., 2000; Tominack *et al*., 1991) and bleeding from the upper gastrointestinal tract may occur. In severe cases hypotension and metabolic acidosis are prominent. The cause of the cardiotoxicity seen is obscure. Respiratory or renal failure may supervene and consciousness may be impaired. A polymorph leucocytosis is common. The radiographic changes of pneumonitis may be present and hypoxaemia may occur. Toxicity to the lung after aspiration of the product may be caused by the surfactant (Martinez and Brown, 1991). It has been suggested that the trimethylsulphonium salt is more toxic than the other salts in humans (Sørensen and Gregersen, 1999).

Management

Treatment comprises supportive measures including those for the management of respiratory and renal failure. Intravenous replacement of lost fluids is indicated. Glyphosate is readily removed by haemodialysis and resin haemoperfusion but not by charcoal haemoperfusion (Tominack, 1999) but the benefits of using these techniques are not established.

Reference dose

Glyphosate has a JMPR ADI of 0.3 mg/kg bw, as the sum of glyphosate and aminomethylphosphonic acid (AMPA), a plant metabolite. This was based upon a long-term rat study in which no definitive treatment-related effect was seen.

In glyphosate-resistent GM crops, AMPA becomes a major metabolite. AMPA is a compound of very low acute toxicity, is non-mutagenic, and few organ-specific effects have been observed in subchronic or chronic studies. The JMPR has allocated this compound a separate ADI of 0.3 mg/kg bw; this was based on the same study as the ADI for glyphosate, both chemicals having similar toxicological profiles.

Glufosinate

Chemical identification

Class: phosphinic acid derivative
Structural formula: see Figure 7.10
Molecular weight: glufosinate, 181; glufosinate-ammonium 198
IUPAC name: 4-[hydroxy(methyl)phosphinoyl]-DL-alanine
CAS name: (±)-2-amino-4-(hydroxymethylphosphinyl)butanoic acid
CAS no.: glufosinate, 51276-47-2; glufosinate-ammonium, 77182-82-2

Glufosinate ammonium is a non-selective phosphinic acid herbicide that inhibits glutamine synthetase in plants and, to some extent, in experimental animals, notably in the kidney (FAO/WHO, 1992). Glutamine synthetase in mammals is involved in ammonia homeostasis in many organs and the glutamine–glutamate shunt between γ-aminobutyrate and glutamate in the central nervous system. However, the enzyme is normally working at a small fraction of its capacity, and considerable inhibition is required in mammals before blood ammonia levels increase (FAO/WHO, 2000).

Animal studies

By mouth in experimental animals glufosinate is poorly absorbed. Penetration of the blood–brain barrier is limited and about 30 per cent of an administered dose is reported to be metabolized. In subchronic studies in rats and mice effects were seen on kidney weights. Central nervous system excitation was seen in dogs. Glufosinate

Figure 7.10 Glufosinate

is not carcinogenic, teratogenic, or genotoxic (FAO/WHO, 1992). At high doses retinal atrophy is seen in laboratory rodents (FAO/WHO, 2000). On the basis of studies in the mouse, it has been suggested that the convulsions are mediated through *N*-methyl-*D*-aspartate receptors (Matsumura *et al*., 2001).

Effects in humans

In high dosage, glufosinate initially causes gastrointestinal symptoms but, later, neurotoxicity in the form of tremor proceeding to convulsions predominates (Hirose *et al*., 1999; Tanaka *et al*., 1998; Watanabe and Sano, 1998).

Reference doses

An ADI of 0.02 mg/kg bw was allocated by the 1991 JMPR (FAO/WHO, 1992; see also FAO/WHO, 2000). This was based upon a long-term rat study, with increases in kidney weight at higher doses.

Defoliants and dessicants, and plant growth regulators

A number of substances are used as defoliants and dessicants in agriculture: sulphuric acid to destroy potato haulms and two closely related trialkylphosphorothioates (DEF and merphos) to defoliate cotton. A notable feature of the latter is that they produce OPIDP in hens (Baron and Johnson, 1964). Chlormequat, used as growth regulator on fruit trees, is a partial cholinergic agonist and its toxic effects in experimental animals reflect that property (FAO/WHO, 2000).

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Part III

Special Types of Pesticide

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8 Microbial Pesticides

Ian C. Dewhurst

Introduction

Microbial pest control agents (MPCAs) are plant protection products that have a micro-organism as the active substance. The micro-organism can be a bacterium, fungus, virus, protozoon, microscopic nematode, or microsporidium. The broader class of biopesticides also includes natural products with pesticidal activity, pheromones, pesticidal compounds produced in plants following genetic modification, and multicellular organisms that are predators or parasites of pests. This chapter restricts itself to MPCAs. Some pesticidal molecules produced by micro-organisms have been purified and are used and regulated in a manner similar to chemical pesticides, e.g. spinosad and abamectin. An extensive compilation of basic information on biopesticides can be found in Copping (1998).

The vast majority of pesticides used to protect plants are based on synthetic chemicals. Concerns arising from the use of chemical pesticides and the removal of a number of chemical pesticides from the market has led recently to moves towards the development and commercialization of more MPCAs. The first MPCAs are reported to have been introduced commercially in the 1930s (Jarvis, 2001). The extent of use and registration of MPCAs varies greatly between countries. Out of over 300 active substances approved for uses in the United Kingdom, only four are based on MPCAs (PSD, 2000). In the European Union (EU), of 722 active substances in use in 1993, only 23 were MPCAs (Table 8.1; EC, 2002a), since then applications have been submitted for six additional MCPAs (EC, 2002b). In the United States, 78 organisms are registered for use as MPCAs (USEPA, 2002a).

The majority of MPCAs have a specific action against a limited range of pests resulting in limited use often in niche markets. With the exception of *Bacillus thuringiensis* (*Bt*) based products there is reported to be limited use by growers (Zahodiakin, 2002) and, in the period 1997–1999, *Bt* products represented approximately 88 per cent w/w of the MPCAs applied in California (Rubin, 2001). Possibly because of the limited use and the fact that to gain approval they should be benign, there is little information in the public domain on the toxicity, infectivity, or pathogenicity to mammals of most MPCAs, the exception being *Bacillus thuringiensis*.

Table 8.1 Microbial pest control agents registered in the countries of the EU on 25 July 1993

	Use	FI	S	DK	IR	UK	NL	B	L	D	AU	F	ES	P	I	EL
Agrotis segetum granulosis virus	in			x												
Aschersonia aleyrodis[a]	in															
Bacillus spaericus	in											x				
Bacillus thuringiensis	in	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Bacillus thuringiensis subsp. *aizawai*	in							x	x							
Bacillus thuringiensis subsp. *israelensis*	in														x	
Bacillus thuringiensis subsp. *kurstaki*	in							x				x			x	
Bacillus thuringiensis subsp. *tenebrionis*	in											x			x	
Beauveria bassiana	in			x								x	x			x
Beauveria basiana bassiana[a]	in															
Beauveria basiana brongniartii[a]	in															
Cydia pomella granulosis virus	in						x	x		x	x	x	x		x	x
Mamestra brassica nuclear polyhedrosis virus	in											x				
Metarhizium anisopliae	in									x	x					
Neodiprion sertifer nuclear polyhedrosis virus	in	x			x											
Phlebiopsis gigantea	fu	x	x	x		x										
Streptomyces griseoviridis	fu	x	x	x												
Tomato mosaic virus	vi															
Trichoderma harzianum	fu		x	x												
Trichoderma polysporum	fu		x	x												
Trichoderma viride	fu		x	x												
Verticillium dahliae Kleb.	fu						x									
Verticillium lecanii	in	x	x	x	x	x	x									

Key: FI – Finland; S – Sweden; DK – Denmark; IR – Ireland; UK – United Kingdom; NL – Netherlands; B – Belgium; L – Luxembourg; D – Germany; AU – Austria; F – France; ES – Spain; P – Portugal; I – Italy; EL – Greece.
x – registered; in – insecticide; fu – fungicide; vi – viricide; [a]notified but not confirmed.
Reference: http://europa.eu.int/comm/food/fs/ph_ps/pro/eva/existing/exis02b_en.pdf

This chapter describes the regulatory processes for MPCAs and summarizes the toxicity and pathogenicity information on a number of MPCAs.

Regulatory approaches

Organisms used as MPCAs are generally considered to be inherently less harmful than chemical pesticides, because they are targeted to act against a specific, often very limited, range of pests and have limited persistence. Though this may be true in particular cases, the initial assumption must be that any biological pesticide has the *potential* to present a significant risk to humans. Naturally produced molecules can be extremely toxic: nicotine or *Clostridium botulinum* toxins are, respectively, of similar or greater acute toxicity than the most acutely toxic synthetic pesticides. Organisms such as *Plasmodium falciparum*, *Escherichia coli* 0157, or *Listeria* spp. have produced significant numbers of deaths or cases of disease in humans. There is therefore a need for those regulating pesticides to *consider* and assess the potential of MPCAs to adversely affect human health.

Microbial pesticides have the ability to multiply and secrete secondary metabolites after application and can present the regulator with more complex challenges than those associated with most chemical pesticides. There is also the issue of study protocols. Test guidelines have been produced by the United States Environmental Protection Agency (USEPA, 1996). However, the internationally accepted test guidelines such as those produced by the Organization for Economic Co-operation and Development (OECD) are designed for testing chemicals and are not readily adapted to the study of micro-organisms.

In a 1981 article, the World Health Organization noted that MPCAs presented different issues and risks to synthetic chemicals and proposed a strategy for determining the 'toxicity' of MPCAs. Regulatory frameworks have been devised by the EU (EC, 2001) and the United States (USEPA, 2002b). The US system was described by Rubin (2001). The EU scheme outlined below also uses a tiered approach to the provision of data and their assessment but there are a number of differences between the requirements. In addition to human health risk assessments, efficacy and environmental impact assessments are performed.

In the EU, the regulation of micro-organisms used in plant protection products falls under the scope of the Plant Protection Products Directive 91/414/EEC (EC, 1991). This legislation requires competent authorities in EU member states to use rigorous standards when evaluating all pesticides, including those based on micro-organisms. For the purposes of the Directive, 'micro-organism' has been defined to apply to, but is not limited to, bacteria, fungi, protozoa, viruses, and viroids; but there is no definition of when a multi-cellular organism is no longer 'microscopic'.

The basic requirements (tier 1) consist of general information on the organism and specific data on toxicity, pathogenicity, and infectivity. The general information

covers the micro-organism's basic biology including its species, strain, natural occurrence, antibiotic resistance, and life cycle; relationship to known pathogens; and the selection/isolation procedures used and whether it has been subject to genetic modification or any techniques which enhance the mutation rate. If the organism has been subjected to extreme selection pressures or induced mutation, then the differences from wild-type organisms of the same strain must be defined. If the mode of action is by the production and secretion of secondary metabolites, it is likely that a more extensive data package will be required to support approval. The production techniques and quality control procedures need to be defined. Relatively minor changes in fermentation conditions can result in significant alterations in the final product and it is essential that tight controls exist to minimize the production of unwanted secondary metabolites and the potential for contamination by other organisms. Limits will be set for contaminating micro-organisms known to be infective/pathogenic to humans.

If there is evidence of extensive human exposure to the organism without adverse effects, then there might be no need to perform animal studies. Therefore the most significant area to be covered is medical surveillance of persons exposed to the organism, with particular emphasis on sensitization reactions.

The data requirements for the human risk assessment are intended to address exposures whilst applying the pesticide, after application, and to those consuming the treated crop. Information of relevance to an assessment of risks to health can be provided without the need to perform specific tests. The following aspects must be addressed: (a) the toxicity, pathogenicity, and infectivity following single exposures via oral, inhalational/intra-tracheal, and intra-peritoneal/subcutaneous routes (an essential element is the rate of clearance of the organism from the body); (b) the genotoxicity (gene mutation and clastogenicity) of exotoxins and lysed cells; and (c) the toxicity, pathogenicity, and infectivity following repeated exposures (28 days). Investigations for sensitization are not mandatory as there is a default assumption that all MPCAs are sensitizers. For intra-cellular replicating organisms the potential to replicate in human cells and interact with the genome must be addressed. Skin and eye irritancy data on the product to be sold can provide information on whether the organism can produce local effects. In the US scheme, genotoxicity and repeated dose studies are part of the second tier (USEPA, 2002b).

Information on likely levels of the organism on treated crops at harvest is required to permit an assessment of the risks to those handling or consuming the produce. This assessment is often more complex than that performed for a chemical pesticide. With a chemical, the levels of residue normally will decrease with time and the degradation of the active substance can be followed relatively simply. A viable biological pesticide may well grow or multiply following application and there may also be morphological or biochemical changes in response to different environmental conditions. If residues of secondary metabolites are detected, then the risk associated with exposure to these will need to be assessed.

The second tier of the EU scheme is performed on a case-by-case basis to address the potential to induce chronic effects, carcinogenicity, *in vivo* genotoxicity, and effects on reproduction if the information at tier 1 indicates there might be long-term health risks associated with the MPCA. The data requirements in the second and third tiers of the US scheme have clearly specified triggers (USEPA, 2002b). It is unlikely that MPCAs with adverse findings in the first tier will be developed further as the costs of performing the studies required at tier 2 will be difficult to recoup.

Toxicity of particular organisms

Ampelomyces quisqualis

Ampelomyces quisqualis is a parasite of powdery mildews and has been used to protect grapes. The spores present in the formulated product are believed to germinate only in the presence of the mildew mycelia. Oral administration of 5×10^9 spores/kg bw to rats produced no evidence of toxicity or pathogenicity. Two hours after rats received an oral dose of 8×10^8 spores/animal no viable spores were detected at any site, including the digestive tract. Repeat administration of 2×10^8 spores per animal per day for 90 days produced no adverse effects in groups of Sprague Dawley rats. An intra-tracheal study reported no adverse effects but was poorly performed. There is no evidence in the published literature that *A. quisqualis* is pathogenic or produces toxins. No cases of allergic reactions were reported amongst operators that had been using *A. quisqualis* for 6 years in the United States (SCP, 2001)

Bacillus thuringiensis

Bacillus thuringiensis (*Bt*) is a spore-forming, aerobic, gram positive bacillus closely related to *B. cereus* an organism which has been associated with human infections. *Bt* can be differentiated from *B. cereus* in that *Bt* produces crystalline protein inclusion bodies in its spores. In other respects *Bt* and *B. cereus* are so closely related that some workers consider them to be the same species. Using 16S rRNA probes, Giffel (1997) showed that 6 of 20 isolates previously identified as *B. cereus* by classical biochemical techniques were more correctly *Bt*. In 2 of the 6 cases, the samples were obtained from food poisoning cases and investigations using Western blotting confirmed enterotoxin production. The sero-types of these isolates were not identified in the report and it is not known whether they were strains used as MPCAs. Jackson *et al.* (1995) also reported the close links between *B. cereus* and *Bt* as part of an investigation of an outbreak of gastroenteritis. Strains previously identified as *B. cereus* were, on more detailed investigation, found to be *Bt*, indicating that cases of illness attributed to *B. cereus* may actually be linked to

strains of *Bt*. They also found that all the *Bt* isolates associated with the outbreak and 20 others were positive when tested for cytotoxicity against a cell culture monolayer (Jackson *et al.*, 1995). The sero-types of the isolates were not identified. The properties of the different strains of *Bt* differ and it is important to relate data to specific strains of *Bt* wherever possible.

Protein crystals produced by *Bt* are responsible for the insecticidal activity and have been identified by the term δ-endotoxin. The δ-endotoxin is coded for by plasmids that can be exchanged with other bacilli. Different strains of *Bt* have activity against different classes of insects and this activity can be related to differences in crystal shape – the different crystals are identified by the code 'Cry #'. The δ-endotoxin in *Bt* spores is in the form of a pro-toxin that becomes active only after it is ingested, solubilized by the alkaline conditions, and activated by enzymes, in the insect gut. The C-terminal of the protein binds to receptors on the gut epithelial cells, is variable, and provides species specificity. The N-terminal is conserved between strains of *Bt* and produces a pore in the gut epithelial cells, leading to cell lysis and death. The protein crystals are reported to be readily degraded by ultraviolet (UV) light, though a wide range of half-lives have been estimated – a few hours to 10 days (IPCS, 1999). Work to develop plants that can synthesize *Bt* δ-endotoxins should permit the production of insecticidal activity without the potential risks associated with the use of live micro-organisms.

Some strains of *Bt* also produce other toxins. The 'β-exotoxin' is a heat stable nucleotide that inhibits RNA polymerases and is toxic to most animal phyla, including insects and mammals. An enterotoxin similar to that produced by *B. cereus* is thought to be responsible for producing diarrhoea and vomiting in mammals. Several other poorly characterized toxins have also been reported (IPCS, 1999). The *Bt* strains supplied for insecticidal use are reported to be β-exotoxin negative and are tested for this on a batch basis. Assays for β-exotoxin look for abnormal mouth part development in house flies, with techniques such as gene probes, *in vitro* cytotoxicity assays, or antibodies currently being investigated.

In addition to the animal studies on toxicity, pathogenicity, and infectivity, a range of studies have been performed on *Bt* strains used as MPCAs. These indicate that use of *Bt* as a pesticide is without significant risk to human health:

1. A review of data on *Bt* by the International Programme on Chemical Safety (IPCS, 1999) cites data on commercial preparations of *Bt* showing that administration (by oral, inhalational, or parenteral routes) of $>10^7$ colony forming units (CFUs)/animal did not produce death or evidence of pathogenicity or infectivity. Mice that had been immunosuppressed by corticosteroids or thymectomy were not killed by an i.p. administration of $\sim 10^7$ spores of *Bt israelensis* (H14) and showed evidence of a slow but sustained clearance of the organism. Artificially activated crystal protein from *Bt israelensis* was lethal when injected into mice and was cytolytic against human erythrocytes, mouse fibroblasts, and pig lymphocytes (IPCS, 1999). A review of

data available to the USEPA was published by McClintock, Schaffer, and Sjoblad (1995). This confirmed the generally safe nature of *Bt* with no infectivity or mortality seen at exposures in the region of 10^7 spores by various routes. When several isolates of *Bt kurstaki* and *Bt israelensis* (including those registered as pesticides) were administered i.p. to mice at 10^8 spores/animal, mortality varied from 0 to 100 per cent depending on the isolate. A report of mortality following i.p. injection of 3.4×10^7 CFUs of *Bt israelensis* into athymic mice may be preparation specific as a different commercial formulation produced no deaths when administered i.p. to athymic or euthymic mice at 2.5×10^7 CFUs. A range of studies on different *Bt* strains and life stages showed that toxicity and mortality could be produced by i.p. or intra-nasal administration and that effects appeared to be related to the vegetative growth phase rather than the crystal protein, flagella, or spores. *Bt* preparations had been found to be mild eye irritants but there were no confirmed cases of hypersensitivity (McClintock, Schaffer, and Sjoblad, 1995).

2. Following two seasons of aerial spraying of an area in Oregon to control gypsy moth, an epidemiology study was conducted to investigate any link between *Bt* and infections (Green *et al.*, 1990). The total exposed population was estimated to be 80 000 in the first year and 40 000 in the second year. The study looked at cases of infection and any associated *Bt* isolates. All samples taken by four large clinical laboratories in the spray area for 1 month after spraying were included. Out of a total of 95 bacillus positive cultures, 55 contained *Bt*. Of these, 52 were considered to have been associated with contamination of skin, tissue, surfaces, or plates. The criteria for exclusion were set in advance and included: no clinical evidence of infection, inappropriate response to antibiotics, presence of other pathogen, or timing. In three cases it was not possible to dismiss the presence of *Bt* as being involved in the case, but there were also confounding factors which prevented firm conclusions being drawn. In all three cases the immune system might have been compromised, associated with known medical history.

3. An epidemiological investigation was performed to monitor any health effects relating to the widespread use of *Bt* to eradicate the Asian Gypsy moth in British Columbia, in 1992. The product used contained *Bt kurstaki* (H3a3b). Both aerial and ground spraying techniques were used in four operations between mid-April and July. The study is slightly compromised as few comparative data were obtained prior to spraying and 'cases' were classed by post-code of residence rather than location at time of spraying. The investigators covered an extensive range of parameters for the main spray zone and surrounding areas: 26 000 calls to a phone hotline; 1140 GP cases from 24 sites; 3500 casualty admissions (10 per cent of the total number of cases in 6 hospitals); 120 ground spray workers; 429 bacterial isolates from hospital samples, and a range of food samples.

There was an increase in symptoms in exposed ground spray workers consistent with exposure to an irritant (consistent with the known properties of the formulation): eye, nose and throat irritation, dry skin, and chapped lips. There was no increase in gastrointestinal problems nor in days off work. Measurements of breathing zone atmosphere of sprayers detected *Bt* spores in the range 2×10^5 to $1.6 \times 10^7/m^3$ giving an estimated maximum exposure over the entire operation of 7×10^8 spores. Nasal swabs were positive for 104 of the 112 workers sampled. Although clearance was generally rapid, some of the highly exposed workers still had *Bt* positive swabs after 4 weeks.

Results from the phone line, GP cases, and casualty admissions produced nothing indicating a clear effect of exposure to *Bt kurstaki*. Cases seen by General Medical Practitioners (GPs) and casualty departments included people from the spray area with respiratory/allergic complaints expected from exposure to Foray 48B. However, there was no clear link between days when *Bt* was sprayed nor residence in the spray zone, with a similar pattern of complaints seen in the ‘control’ groups not resident in the main spray zone. Swabs taken by GPs showed 10–15 per cent of samples to be positive for *Bt kurstaki* with a higher frequency in the spray zone. Of the 429 bacterial isolates from hospital samples obtained from a wide range of tissues, fluids, and intravascular lines, 325 contained *Bt kurstaki* but only 43 contained it in isolation. In none of the cases did the presence of *Bt kurstaki* meet predetermined criteria indicating it was associated with infection or disease though there was an indication of a weak link to genitourinary infections and eye contamination. Food and environment samples indicated *Bt kurstaki* was present in the environment independent of the spray programme, as positive and negative samples were found in produce from the same stores independent of site of production and time of sampling (up to 6 weeks after the spraying operation).

The authors concluded that although there was clear evidence of exposure to *Bt kurstaki* in both workers and the general population, there was no case indicating pathogenicity or infection. The authors noted the potential for immunosuppressed people and those with HIV to be at extra risk but did not comment specifically on whether such people were present in the study population (Noble, Riben, and Cook, 1992).

4. An operation to spray Foray 48B (containing *Bt kurstaki* H3a3b) from aircraft over an area of New Zealand to control Tussock moth in 1996/97 was studied prior to a follow-up operation. Monitoring of casualty attendances, miscarriages/birth defects, corneal ulcers, phone hotline calls, medical laboratory samples, and hospital discharges was performed in areas in the spray zone and those bordering it. Sporadic increases and decreases were noted but no statistically significant results were found. A specific investigation of miscarriages showed similar patterns in treated and non-treated areas and over time but continuing surveillance of birth defects would be undertaken. The authors concluded that there was no evidence that *Bt kurstaki* would produce: (a) Infection or

compromise the respiratory tract; (b) gastrointestinal infection; (c) primary or secondary wound infection; (d) corneal ulcers, or (e) infection or illness in conjunction with other bacteria leading to respiratory infection or miscarriage (Anonymous, 1997).

A number of adverse findings relating to *Bt* have been reported; however, it is not always clear how these reports relate to the actual use of *Bt* strains as MPCAs:

1. A French soldier injured by a land mine was found to have *Bt* sero-type H34 in necrotic soft tissue from the site of injury. Initial studies with this strain in immunosuppressed mice showed it to induce myonecrosis following cutaneous administration (Hernandez *et al.*, 1998). The same group of workers then performed studies with immunocompetent mice (5/group, BALB/c) on cultures of *Bt* H34, *Bt* H12 (obtained from a clinical specimen but considered clinically irrelevant), and two sero-types used in pesticides (H14 and H3a3b) (Hernandez, Cavallo, and Cook, 1999). Following intra-nasal administration (10^5, 10^6, 10^7, or 10^8 spores/animal) mortality, lung pathology, and residual bacterial count were recorded. Haemolytic activity of supernatants from stationary phase cultures was also investigated, using rabbit erythrocytes. With H34, all mice administered 10^8 spores died within 8 h, the lungs showing oedema, alveolar damage, nucleophilic infiltrate, and ulceration. Administration of 10^7–10^5 spores produced no deaths, just a local inflammatory response. Spores of H34 were still present in blood samples 10 days after administration. There were no deaths in animals receiving H12, just an inflammatory response. Administration of 10^8 spores of H14 produced deaths in 40 per cent of the mice, while 80 per cent of animals receiving 10^8 spores of H3a3b died; there were no deaths at lower inoculation rates. Lesions were similar to those produced by H34. The greatest haemolytic activity was seen with H34 (titre = 1:256); for H12, H3a3b, and H14, the titres were 1:2, 1:128, and 1:32, respectively. The haemolytic activity was associated with a heat labile (70°C for 10 min) fraction of >30 kDa molecular weight similar to a *B. cereus* haemolysin. The nature of a haemolysin produced by *Bt kurstaki* was investigated and found to be identical to a haemolysin derived from *B. cereus* HG-6A associated with a case of food poisoning (Honda *et al.*, 1991). The authors note that the exposures used in this study are very high compared with likely exposures from pesticidal use but show the two pesticidal strains to produce a haemolytic toxin with the potential to produce lung lesions. No information has been found relating to the potential for *Bt* strains to produce toxins under typical conditions associated with pesticidal use.

2. Four strains of *Bt* (*Bt israelensis* ONR60A and O2-72; *Bt kurstaki* HD1 and HD73) closely related to those used in pesticide formulations were found to give positive results in tests for *B. cereus* enterotoxin. All four strains expressed no or minimal haemolytic activity (Carlson, Caugant, and Kolsto, 1994).

3. Perani, Bishop, and Vaid (1998) used larval toxicity, the polymerase chain reaction (PCR), electrophoresis, latex agglutination, and ELISA to study 48 *Bt* isolates, obtained from soil, for production of β-exotoxin, diarrhoeal enterotoxin, and δ-endotoxin (Cry 1B gene). The aim was to identify strains that produced the δ-endotoxin without the other two toxins. None of the isolates met this criterion. Approximately half the isolates were positive for exotoxin and approximately 80 per cent positive for the enterotoxin. Analysis of the frequency of appearance of the toxins indicated that the genes coding for them were probably ($p < 0.05$) not linked. A conclusion of the paper was that the larval bioassay for β-exotoxin is of most relevance to human safety as there were different types of this toxin. A test specific for only one type may provide false negative results.

4. Investigations into the levels of *Bacillus* diarrhoeal enterotoxin in one strain of *B. cereus* (positive control) and 10 strains of *Bt* (one pure strain HD-1; an isolate form a case of bovine mastitis and eight commercial preparations). The enterotoxin titres in *Bt* strains varied between 15 and 242, with the *B. cereus* strain having a titre of 1629. An immunoassay for the enterotoxin showed it to be degraded by heating at 100°C for 12 min. The author expressed concerns over the validity of the testing performed on *Bt* products, which were performed on spores rather than the vegetative forms which can produce toxins (Damgaard, 1995). This is a valid point and applies to all MCPAs that are formulated as spores. Subsequently the same group isolated a number of *Bt* strains from food items. The isolates included strains used in pesticidal products but it was not possible to confirm whether contamination was due to natural occurrence or pesticidal treatment of raw materials (Damgaard *et al.*, 1996). Damgaard *et al.* (1997) reported the presence of four strains of *Bt* in infections in burn wounds and from fluid used to treat the burns. None of the strains had insecticidal activity nor any H-serotype as they were non-flagellated. It is not clear whether these strains of *Bt* were involved in producing the infection or were merely present as contaminants.

5. The potential use of *Bt kurstaki* δ-endotoxin (Cry1Ac) as an adjuvant in vaccination was investigated by Vazquez-Padron *et al.* (1999). These workers showed that i.p. or gavage administration of 100 µg δ-endotoxin/mouse in solution or as crystals could increase anti-Cry1Ac immunoglobulin titres in serum, faeces, or the gut. Exposures to lower doses (<10 µg/mouse) did not produce any consistent pattern or statistically significant increase in antibodies. However, it is not clear whether the findings were due to anything other than a general response to a foreign protein as control mice administered bovine serum albumin (BSA) were investigated for anti-Cry1Ac immunoglobulins and not anti-BSA. When cholera toxin was administered to mice in conjunction with either BSA or Cry1Ac, the anti-cholera toxin titres were similar irrespective of the co-administration (no results are presented for administration of cholera toxin alone). The absence of control data in the paper makes it impossible to put

the immunogenic activity of *Bt kurstaki* δ-endotoxin (Cry1Ac) into context. The authors concluded that *Bt kurstaki* δ-endotoxin (Cry1Ac) is a potent systemic and mucosal immunogen and its ingestion may potentially produce a response typical of mucosal inflammatory diseases such as Crohn's disease, though the basis for this conclusion is uncertain. This finding could be of significance if the gene for Cry1Ac protein is introduced into plants.

The majority of data available confirm pesticidal strains of *Bt* to be non-pathogenic, non-infective, and of low toxicity, though sporadic effects have been associated with individual preparations. The latter highlights the need for reliable quality control procedures to be operative. Epidemiology studies following widespread aerial spraying of *Bt* in Canada and New Zealand did not find any clear evidence of adverse effects. A number of reports indicate that some strains of *Bt*, including some used as pesticides, have the potential to produce toxins in addition to the δ-endotoxin associated with insecticidal activity. These other toxins can produce symptoms typical of *B. cereus* poisoning (diarrhoea and vomiting). The toxins are reported to be produced by vegetative stages rather than the spores used in studies on commercial *Bt* products, but the relevance of this to human exposures needs to be clarified. Traditional investigations of food poisoning organisms would not differentiate between *Bt* and *B. cereus* raising the possibility that cases previously attributed to *B. cereus* could be related to *Bt*. There are clear differences between different strains/isolates of *Bt* reinforcing the need for those producing *Bt*, and other MPCAs, to adopt adequate quality control procedures to ensure integrity of the organism.

Beauveria bassiana

Beauveria bassiana ATCC 74040 has activity against a wide range of insect pests, possibly involving the production of beauvericin. Intra-peritoneal administration to rats of 2×10^7 CFUs/animal produced no evidence of pathogenicity, infectivity, or toxicity. No animal mortality, overt toxic effects, or evidence of pathogenicity were noted in rats dosed orally with 1.9×10^8 CFUs/animal or intratracheally at 2.5×10^9 CFUs/animal nor in rabbits treated dermally with about 8×10^7 CFUs/animal. The time to clear the intra-tracheal and intra-peritoneal doses was less than 15 days. An inflammatory response was noted in the lungs of animals dosed intra-tracheally but this is considered to be a normal reaction to the presence of large titres of foreign material. A formulation based on *Beauveria bassiana* ATCC 74040 was found to be a skin sensitizer although no hypersensitivity incidents have been reported (USEPA, 2000).

Coniothyrium minitans

Coniothyrium minitans is a ubiquitous fungus with the ability to control *Sclerotinia*. *C. minitans* mycelial growth is halted at temperatures above 33°C with no

germination at 30°C. Oral or dermal dosing of 2×10^8 CFUs/animal or the intraperitoneal injection of 2×10^7 CFUs/animal produced no deaths or evidence of pathogenicity in rats. Nose only exposure to the formulated product at 12.7 mg/L for 4 h produced no abnormal clinical signs or gross changes, although the particle size (mass median aerodynamic diameter of >23 μm) meant only a small proportion of the dose would be respirable. There are no reports of *C. minitans* exhibiting toxicity or pathogenicity in mammals (USEPA, 2001), as would be anticipated from its sensitivity to temperatures >30°C.

Granulosis viruses and nuclear polyhedrosis viruses

These two groups of viruses are members of the family of baculoviruses. These are complex viruses that are protected by a protein overcoat forming an occlusion body. A number of these, including *Neodiprion sertifer nuclear polyhedrosis virus, Cydia pomella granulosis virus*, and *Mamestra brassica nuclear polyhedrosis virus* have insecticidal uses, primarily against moths. Susceptible insect larvae ingest the virus, which becomes active only after ingestion of the occlusion bodies. In the larval gut, the protein overcoat quickly disintegrates, and the viral particles proceed to infect digestive cells and interfere with the function of several larval organs, including food absorption in the gut. Larvae die after a few days. These viruses occur naturally and are considered to present no known risks to humans (USEPA, 2002c).

Phlebiopsis gigantea

Phelbiopsis gigantea is a *Basidomycete* that grows sapprophytically on decaying wood and is used to control *Heterobasidion annosum* on felled tree stumps (*H. annosum* can infect live trees). The mechanism of action is reported to be competition for nutrients and not to rely on the secretion of secondary metabolites. There are no reports of adverse reactions in humans exposed to it from natural growth in woodland or in operators using it over approximately 1000 man-years. *P. gigantea* produced no toxicity when fed to mice and hamsters. Granulomatous and inflammatory lesions containing hyphal elements were seen in hamsters that received subcutaneous injections of *P. gigantea*, although no viable forms were found in cultures of excised organs. *P. gigantea* does not grow actively at 35°C and is killed at 38°C. No indication of hypersensitivity or ill health was seen in the individual that had been formulating the product for 30 years (PSD, 1998).

Pseudomonas chloraphis

Pseudomonas chloraphis MA342 has activity against fungi that attack crop roots and can be used to protect cereal seed. The precise mechanism of action has

not been identified but is believed to involve the secretion of an iron-chelating protein and the production of 2,3-deepoxy-2,3-dihydrorhizoxin (DDR). *Pseudomonas chloraphis* MA342 is not toxic or pathogenic to rats via the oral route (2×10^{12} CFUs/kg bw) or inhalation route (5×10^{5} CFUs/rat) and is not closely related to any organisms considered to be pathogenic to humans. The metabolite DDR has been found to be a potent *in vitro* aneugen, inducing a significant increase in c-mitotic cells at concentrations of ≥ 25 pM in Chinese hamster V79 cell cultures. This aneugenicity was reproduced *in vivo* with oral doses of 2 and 18.6 mg/kg bw producing increases in micronucleated polychromatic erythrocytes in NMRI mice (SCP, 2002). The risks associated with the use of *Pseudomonas chloraphis* MA342 and the production of DDR are uncertain, but might be low as the DDR is reportedly only produced on the germinating seed.

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9 Biocides

Bryan Ballantyne and **Susan L. Jordan**

Introduction

Industrial antimicrobials, or biocides, are used in many aqueous industrial processes and formulations. Industrial biocides differ from medical antibiotics in that they are generally less specific and hence less likely to induce bacterial resistance. These industrial chemicals require approval of the Environmental Protection Agency (EPA) for use in the United States, and are regulated by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), and require extensive chemical, efficacy, and toxicological testing before approval. Many industrial processes, such as pulp and paper manufacturing, use large amounts of recirculated water which, if left untreated, will become contaminated leading to odours, equipment fouling, and failure and contamination of the finished product. Aside from general contamination, bacteria in untreated systems can attach to equipment surfaces. The initial interaction of the bacteria with the surface is reversible, but eventually becomes irreversible due to adhesion of exopolysaccharide polymers (Costerton *et al.*, 1987). The attached bacteria form a biofilm on the surface that may cause major problems in a given system such as clogging of equipment, breaking off to move into downstream systems, adding pressure to the system, and slowing down the process. In addition, there can be costly down time associated with cleaning out systems plagued by biofilm. Also, biofilms may tend to harbour anaerobic bacteria under the biofilm layer. These sulphate-reducing bacteria (SRB) cause additional corrosion problems in tanks and pipes, leading to process leaks and potentially dangerous situations. In many cases, the problems created by biofilms (sessile bacteria) are much more serious and detrimental than those caused by free floating (planktonic) bacteria. Sessile and sulphate-reducing bacteria, however, are typically more difficult to kill than planktonic bacteria. This is due, in part, to transport limitations into the biofilm and potentially phenotypic changes in bacteria, and making the bacteria more resistant to certain biocides (Costerton *et al.*, 1987; Grobe and Stewart, 2000). Accordingly, industrial biocides vary in their ability to control these bacteria.

Industries that use biocides include oil field water flooding, pulp and paper mills, cooling towers, retort processing systems, waste water treatment, and textile

processing. These systems are dynamic in nature with periodic turnover of the treated water that requires a sump-side addition of the biocide. Biocides used in these systems do need to sterilize the systems, but must be able to reduce microorganism load and maintain low enough levels to help prevent biofilm formation. Some biocides are added continuously at low doses to maintain control, while others are added as slug doses (higher concentrations added quickly with repeated additions over time). The type of addition will depend on the system being treated as well as the biocide.

Aside from treating dynamic systems, biocides are also used to preserve formulations used for personnel care products (e.g. shampoos and cosmetics), detergents (e.g. fabric softeners), and industrial applications (e.g. concrete admixtures, paints, adhesives, and lubrication fluids). When used as a preservative, a biocide must have long-term activity as it is added only once to the product and is intended for long-term storage and repeated insult. Preservatives used in these applications include *iso*-thiazolones, glutaraldehyde (GA), 2-bromo-2-nitropropane-1,3-diol (BNP), *ortho*-phenylphenol, formaldehyde, and formaldehyde-releasing compounds, carbamates, and parabens.

Sanitizers, disinfectants, and sterilants represent another class of biocides. Many countries have specific criteria that must be met to qualify for these labels. The level and type of organism reduction is important. In general, sterilants are required to completely kill bacterial spores, which aside from prions, are considered amongst the most difficult of organisms to kill followed by *Mycobacterium*, non-lipid or small viruses, fungi, vegetative bacteria, and lipid or medium sized viruses. The Centers for Disease Control and Prevention (CDC, Atlanta, Georgia, US) classifies sterilization and disinfection processes using liquid chemicals as follows:

- Sterilization: sporicidal chemical, prolonged contact (e.g. GA, chlorine dioxide, hydrogen peroxide, peracetic acid).

- High-level disinfection: sporicidal chemical, short contact time (e.g. GA, chlorine dioxide, hydrogen peroxide, peracetic acid).

- Intermediate-level disinfection: some tuberculocidal activity (e.g. phenolics, iodophors, or chlorine compounds).

- Low-level disinfection: no tuberculocidal activity, kills most bacteria and viruses quickly, can include sanitizers (e.g. quarternary ammonium compounds, some iodophors, and some phenolics).

For sterilization, autoclaving is typically the method of choice unless the instrument is heat sensitive. In that case ethylene oxide (EO) or gas plasma sterilization is used. The low-temperature liquid chemical sterilants referred to here [GA and peracetic acid (PAA)], are typically used for high-level disinfection rather than full sterilization, even

though both chemicals are sporicidal under certain conditions. High-level disinfectants are used to disinfect semicritical devices which enter the body and may come into contact with mucosal surfaces, but do not, in general, break through these membranes and enter the normally sterile areas of the body. In the United States, high-level disinfectants must be able to kill spores under some conditions and must be able to kill some spores and all mycobacteria under the label conditions.

This chapter will focus primarily on water treatment biocides, notably *iso*-thiazolinones, glutaraldehyde (GA), quarternary ammonium compounds, 2,2-dibromo-3-nitilopropionamide (DBNPA), tetra-(hydroxyethyl)-phosphonium sulphate (THPS), BNP, and methylene-*bis*-thiocyanate (MBT). In addition, GA and PAA will be discussed in the context of sterilization and high-level disinfection.

Chemistry of biocides

Biocides typically are separated into two main classes: oxidizers such as halogens, and non-oxidizers such as *iso*-thiazolones, GA, DBNPA, quarternary ammonium compounds, THPS, BNP, and MBT.

Industrial biocides are chemically diverse compounds and include many classes of chemicals such as phenolics, surface-active agents, aldehydes, halogens, heavy metals, and many other miscellaneous compounds. They can be nucleophiles or electrophiles, oxidizing agents or reducing agents. Because of this diversity there is no general mechanism that can be used to describe the mode of action of these chemicals (Denzer, 1995). While individual metabolic processes may be affected by biocides, the broad spectrum of lethality exhibited by many of the most successful biocides suggests a certain lack of specificity. For example, GA is known to non-specifically react with amine and thiol groups of cellular and membrane proteins and enzymes. Phenolics interact with the cell wall, interfere with membrane potential, and react with thiols and amines. Surface-acting agents, such as quarternary ammonium compounds, affect general membrane permeability (Denzer, 1995; Favero, 1994).

Because of the non-specific nature of the mechanisms of action of the many industrial biocides, microorganisms do not develop genetic resistance to biocides as do organisms to medical antibiotics. There are, however, many instances of organisms developing some degree of resistance or tolerance to particular biocides (Chapman, 1998; Chapman, Diehl, and Fearnside, 1998; Grobe and Stewart, 2000). The mechanism for this resistance presumably differs with each biocide. In some cases it may be opportunistic growth of a tolerant subclass, in others the stress induced by the biocide or inclusion in a biofilm may trigger phenotypic changes in the organism making it more difficult to kill. In any case, the biocide industry has developed strategies to deal with these issues, such as alternating biocides to prevent development of tolerance and using synergistic blends.

Since biocides have many differing mechanisms of action, they also have differing degrees of activity against the organisms of interest. Therefore, not every biocide is

suitable for each application, and in some cases biocides are used together or in succession in order to obtain optimum biocidal activity. Some biocides are primarily used for long-term preservation, whilst others are used for quick kill and short half-life. Some of the more important and widely used biocides are discussed below.

2,2-Dibromo-3-nitrilopropionamide (DBNPA)

Identities and physicochemical properties

Structural formula: see Figure 9.1
Molecular formula: $C_3H_2Br_2N_2O$
CAS no.: 10222-01-2
Chemical family: halogenated cyanoacetamide
Synonyms: 2,2-dibromo-2-cyanoacetamide
dibromo-2-carbamoylacetonitrile
DBNPA
Molecular weight: 241.86 g/mole
Melting point: 123–125°C
Thermal decomposition: >c. 60°C
Vapour pressure: 8.25×10^{-4} mm Hg (25°C)
Specific gravity: 2.375 (21°C)
Appearance: white crystalline powder
Odour: mild antiseptic
Solubility: acetone – 35 g/100 g
ethanol – 25 g/100 g
dimethylformamide – 120 g/100 g
polyethylene glycol – 200–120 g/100 g
soluble in water to 17 g/L (25°C)
K_{ow}: 6.3
DBNPA is available commercially as a powder, or 5–20 per cent solutions containing 40–60 per cent glycol
Sources of information: Clearon Corp. (2000); Paulus (1993, pp. 241–249); Wuzhou Int. Co. (2002)

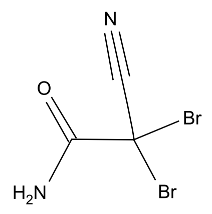


Figure 9.1 2,2-dibromo-3-nitrilopropionamide. CAS no. 10222-01-2

Mechanism of action

DBNPA has been demonstrated to bind to living and dead cells, suggesting it is not transported into cells, but binds to the surface membrane. DBNPA reacts with thiols, and most likely inhibits proteins and enzymes on the cell surface by reacting with key groups within these enzymes. In particular, DBNPA has been shown to interfere with the transport of *o*-nitrophenyl-β-D-glycosidase and ^{14}C-glucose into bacteria (Friend and White Kettle, 1980).

Stability and decomposition

DBNPA is stable at acidic pH, but begins to degrade quickly as the pH is raised ($t_{1/2}$ at pH 6.0 = 155 h; $t_{1/2}$ at pH 9.0 = 0.3 h) (Friend and White Kettle, 1980). DBNPA is not very stable in the presence of organics, nucleophiles, or UV light (Blanchard, Gonsior, and Hopkins, 1986), and is particularly sensitive to thiols and sulfides. DBNPA degrades to dibromoacetamide and ultimately to CO_2, NH_3 and $Br^{\bullet}$. In a secondary pathway, DBNPA is debrominated by UV light to cyanoacetamide (Blanchard, Gonsior, and Hopkins, 1986; Exner, Burk, and Kyriacou, 1973).

Biocidal uses

DBNPA has a broad spectrum of biocidal activity against Gram-negative and Gram-positive bacteria, yeast and moulds. Because of its relatively short half-life in neutral to alkaline formulations, DBNPA typically is used in industrial applications such as cooling towers, pulp and paper processing, water recirculating systems, and reverse osmosis membranes. DBNPA can be added at a low concentration continuous dose (0.5–5 ppm) or at a higher slug dose (5–20 ppm), added periodically as needed for control. Laboratory studies have shown that DBNPA is effective in destroying *Legionella pneumophilia* at concentrations of 10^5 to 10^6 viable cells/ml (Anonymous, 1979; Skaliy *et al.*, 1980).

Toxicology

Acute toxicity

Peroral. DBNPA is of moderate acute peroral toxicity, with an LD_{50} of 308 mg/kg in the rat. Upper alimentary tract corrosive effects may develop. *Percutaneous*. By 24-h occluded contact, DBNPA is of slight acute percutaneous toxicity, with an LD_{50} of >2000 mg/kg in the rat. *Vapour exposure*. A low acute toxicity is reported in the rat with a 4-h LC_{50} of 0.32 mg/m^3 (Clearon Corp., 2000).

Primary irritation

Skin. Undiluted DBNPA is reported as being moderately irritating to the skin (Clearon Corp., 2000). *Eye*. The undiluted material is reported as corrosive to the rabbit eye (EPA, 1994).

Subchronic repeated peroral toxicity

A NOEL of 15 mg kg^{-1} day^{-1} was reported for a 13-week peroral study in the rat (Clearon Corp., 2000).

Chronic toxicity

No information is available.

Oncogenicity

No information is available.

Genetic toxicology

DBNPA is not mutagenic in an Ames test, and is not clastogenic in a chromosomal aberration test with Chinese hamster ovary (CHO) cells or a chromosomal aberration test with human lymphocytes. It did not induce unscheduled DNA repair *in vitro* with rat hepatocytes (Clearon Corp., 2000).

Developmental toxicology

DBNPA is reported as being not teratogenic in rabbits, in which test a NOAEL of 10 mg kg^{-1} day^{-1} was reported for fetotoxicity (Clearon Corp., 2000).

Reproductive toxicology

In a two-generation study with rats, a no observed adverse effect level (NOAEL) for reproductive effects was cited as $\geq$30 mg kg^{-1} day^{-1} (Clearon Corp., 2000).

Human toxicology

By local contamination, DBNPA will produce moderate to severe skin irritation and severe (corrosive) lesions to the eye. Those handling the material should wear appropriate protective clothing and eye goggles. Because of its corrosive properties, swallowing of DBNPA should be regarded as an emergency medical situation, since complications may occur as a result of the development of corrosive lesions to the upper alimentary tract, and there is a potential for an aspiration hazard. Because of these considerations, vomiting should not be induced in cases of ingested DBNPA.

No ACGIH TLV is available.
US Department of Transportation (DOT) Packing Group II, Class 6.1.

Ecotoxicology

Values for aquatic toxicity are as follows:

Rainbow trout	96-h $LC_{50} = 2.3\,mg/L$
Sheepshead minnow	96-h $LC_{50} = 3.4\,mg/L$
Bluegill sunfish	96-h $LC_{50} = 0.72\,mg/L$
Mysid shrimp	96-h $LC_{50} = 0.72\,mg/L$
Eastern oyster	96-h $LC_{50} = 0.37\,mg/L$
Daphnia magna	48-h $EC_{50} = 0.86\,mg/L$

Values for avian toxicity are as follows:

Bobwhite quail	acute oral $LD_{50} = 354\,mg/kg$
Mallard duck	dietary $LC_{50} \geq 5620\,ppm$
Bobwhite quail	dietary $LC_{50} > 5620\,ppm$

Methylene*bis*thiocyanate (MBT)

Identities and physicochemical properties

Structural formula: NCS—CH_2—SCN
CAS no.: 6317-18-6
Molecular weight: 130.13 g/mole
Melting point: 101–105°C
Appearance: yellow powder
Solubility: water – poorly
Sources of information: Paulus (1993, pp. 421–422).

Mechanism of action

MBT interferes with cell metabolism by complexing with iron in enzymes such as cytochromes, and disrupts energy transfer reactions that rely on iron-containing enzymes.

Stability and decomposition

MBT is commercially available as a dilute liquid or in a dry powder form (95 per cent active) (Friend and White Kettle, 1980; Hugo and Russell, 1982). MBT is more stable at acidic pH than at alkaline pH ($t_{1/2}$ at pH 6 = 288 h; $t_{1/2}$ at pH 9 = 1 h), rapidly hydrolysing at alkaline pH to cyanide and thiocyanate (Friend and White Kettle, 1980).

Biocidal uses

MBT has been used as a biocide for over 30 years. It has a broad spectrum of activity against bacteria, yeast, moulds, algae, and anaerobic sulphate-reducing bacteria (Friend and White Kettle, 1980; Wehner and Hinz, 1971). MBT usually is formulated as a liquid in organic solvent or aqueous suspension. It is primarily used in slug doses of 2–10 ppm in cooling tower, pulp and paper processing, and leather applications. Because of its antifungal activity, it is also used, at higher concentrations, as an antisapstain wood preservative for short-term preservation of freshly sawn wood.

Toxicology

Sensitizing potential

Skin. MBT is shown to have a strong sensitizing potential in a guinea pig maximization test (Andersen and Hamann, 1983). Cases of possible allergic contact dermatitis have been described (Jappinen and Eskelinen, 1987).

Subchronic toxicity

Peroral. Male and female Fischer 344 rats and $B6C3F_1$ mice were given MBT by gavage at dosages of 1, 2, 4, 8, or 16 mg/g daily for 5 days/week for 13 weeks. Mortalities occurred at 2, 4, 8, and 16 mg/kg with rats and 8 and 16 mg/kg with mice. Histology demonstrated squamous mucosal hyperplasia and hyperkeratosis in the forestomach, with a NOAEL of 4 mg/kg for male rats and 2 mg/kg for female rats and male and female mice (NTP, 1993a).

Quarternary ammonium compounds (quats)

There are many different biocidally active quats in use. Each may differ in some properties, such as specific efficacy, sensitivity to organic load or salt, and degree of foaming. Quats are available commercially as clear to yellow liquids and are very soluble in water (Paulus, 1993, pp. 375–387).

Mechanism of action

Quats are cationic compounds containing a nitrogen bonded to four alkyl or heterocyclic groups and a small counter ion. In order to have antibacterial efficacy, at least one group must be C_8–C_{18} in length (Hugo and Russell, 1982). Variation in chain length and type can significantly affect biological activity. Quats are considered surface-active agents. The hydrophobic chains are able to orientate themselves in

the cell wall with the hydrophilic portion remaining outside the cell. In this manner, quats disrupt cytoplasm membranes, releasing K^+ ions and other constituents (Merianos, 2001). Quats also dissociate conjugated proteins in the membrane, and in this manner alter membrane permeability.

Stability and decomposition

Quats have varying sensitivity to hard water (salt) and organic load. The extent of inactivation varies with the chain length or substitution on the benzyl ring of *n*-alkyl-*n*,*n*-dimethylammonium chloride (ADAC) (Merianos, 2001). They tend to be more active under alkaline conditions, and less soluble as the hydrophobic chain increases in length. They tend to be inactivated and potentially precipitate in the presence of anionic surfactants (Hugo and Russell, 1982).

Biocidal uses

Quats are more active against Gram-positive bacteria [minimum inhibitory concentration (MIC) of 1–25 ppm] than Gram-negative bacteria [MIC >500 ppm]. Quats are used as hard surface disinfectants in household products, food processing, hospitals, and animal housing. Quats are not normally used as high-level disinfectants or instrument sterilants since they lack mycobacterial and sporicidal activity (Hugo and Russell, 1982; Merianos, 2001; Paulus, 1993). Quats are active against lipophilic viruses and fungi, and are used in pools against algae. In addition to disinfection, quats are used as preservatives in personal care products (e.g. shampoos) as well as household products, and in water recirculating processes and cooling towers. Because quats have a tendency to foam, they require care when applied in large systems. Quats are often used in combination with other biocides for synergistic effects. For instance, a glutaraldehyde/quarternary ammonium formulation is used in cooling towers and pulp and paper processing. Two representative quats are discussed below.

Benzyl-*n*,*n*-dimethyl ammonium chloride (ADAC)

Identities and physicochemical properties

Structural formula: see Figure 9.2
$n = 8–16$
CAS no.: 68391-01-5
Molecular weight: 354 g/mole
Freezing point: +5 to –12°C
Boiling point: 80°C (50%)
Flash point: 34°C
Density: 0.95 g/cm^3
Viscosity: 860 mPa

Figure 9.2 Benzyl *n*-alkyl-*n,n*-dimethyl ammonium chloride. CAS no. 68391-01-5

Di-*n*-decyl-dimethyl ammonium chloride (DDAC)

Identities and physicochemical properties

Structural formula: see Figure 9.3
CAS no.: 7173-51-5
Molecular weight: 362.09 g/mole
Freezing point: −20°C
Flash point: 29°C
Density: 0.91 g/ml
Viscosity: 52 mPa

Toxicology

Sensitizing potential

Skin. A few cases of occupational allergic contact dermatitis have been described (Dejobert *et al.*, 1997).

Ecotoxicology

The following acute toxicity values have been obtained for aquatic species (Farrell *et al.*, 1998):

Acipenser tyransmontanus	24-h LC_{50} = 1–10 ppm
Pimephales promelas	96-h LC_{50} = 0.39 ppm

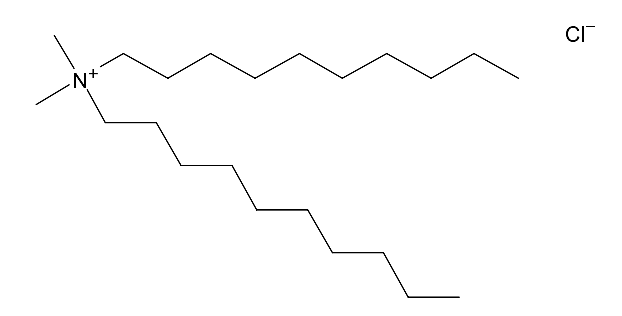


Figure 9.3 Di-*n*-decyl-dimethyl ammonium chloride. CAS no. 7173-51-5

Platichthys stellatus	96-h $LC_{50} = 2.0$ ppm
Daphnia magna	48-h $LC_{50} = 0.037$ ppm
Mysidopsis bahia	48-h $LC_{50} = 0.039$ ppm
Hyalella azteca	48-h $LC_{50} = 0.106$ ppm
Neomysis mercedis	48-h $LC_{50} = 0.947$ ppm

2-Bromo-2-nitropropane-1,3-diol (bronopol; BNP)

Identities and physicochemical properties

Structural formula: see Figure 9.4
Molecular formula: $C_3H_8BrO_2N$
Molecular weight: 200 g/mole
Melting point: 130–133°C
Appearance: white crystalline solid
Aqueous solutions of BNP release 30% formaldehyde over time
Sources of information: Paulus (1993, pp. 55–57, 70–72)

Mechanism of action

Formaldehyde released from BNP is not the active species that kills the microorganisms. BNP is electrophilic in nature, and its activity is due primarily to oxidation of thiols in proteins to form disulphides. BNP also causes membrane damage (Stretton and Manson, 1973). Because BNP activity is seen mainly with dividing cells, the primary effects likely involve metabolic processes. Specifically, BNP has been shown to inhibit the dehydrogenases involved in glucose metabolism located in cell membranes and the thiol-acetylating of CoA (Stretton and Manson, 1973). BNP is active against Gram-positive and Gram-negative bacteria as well as yeast and fungi. The effect is primarily biostatic instead of biocidal, but a change in mechanism may occur. Cell death occurs as the oxidized thiols produce peroxide and super oxide radicals. Cidal concentrations of BNP are typically 10 times those of biostatic (preservative) concentrations (Gutherie, 1997; Shepherd, Waigh, and Gilbert, 1988). The activity increases with increasing pH, from 5.5 to 8.0.

Figure 9.4 2-bromo-2-nitropropane-1,3-diol. CAS no. 52-51-7

Stability and decomposition

Aqueous solutions of BNP are more stable at an acidic than an alkaline pH. As pH increases (>9.0), BNP decomposes, releasing formaldehyde to form bromonitroethanol and, eventually, $Br^{\bullet}$, nitrite and nitro alcohols (Bryce *et al.*, 1978; Challis and Yousaf, 1991; Paulus, 1993, pp. 55–57, 70–72). BNP also loses activity in the presence of thiols. When thiols are added to a formulation containing BNP, they can reverse the effects of BNP on the enzymes (Bryce *et al.*, 1978; Stratton and Manson, 1973).

Biocidal uses

Because of its biostatic properties, BNP typically is used as a preservative in slightly acidic formulations, which include cosmetics, toiletries, and household products, as well as paints and adhesive (Bryce *et al.*, 1978). BNP is particularly effective against Gram-negative bacteria such as *Pseudomonas aerations* (MIC ≈ 50 ppm). It is used at 50–500 ppm against bacteria and 200–2000 ppm against fungi (Paulus, 1993, pp. 55–57, 70–72).

Toxicology

Acute toxicity

Peroral. BNP has a moderate peroral toxicity in the rat, with LD_{50} values in the range 254–342 mg/kg (Eriksson, Johnson, and Tornlund, 1995). *Percutaneous*. In aqueous solution, BNP is of low acute percutaneous toxicity in the rat, having an LD_{50} of >1600 mg/kg (Eriksson, Johnson, and Tornlund, 1995). *Subcutaneous*. By subcutaneous dosing BNP is of moderate toxicity, with an LD_{50} of 200 mg/kg.

Primary irritation

Skin. At 2 per cent and above BNP is a severe skin irritant. *Eye*. BNP causes severe eye irritation at 5 per cent and above.

Sensitization

Skin. Animal studies indicate that BNP has a skin sensitizing potential. Controlled human studies and cases of occupational exposure show that BNP can cause allergic contact dermatitis. Although some studies suggest that BNP is a common cause of skin sensitization (Peters, Connolly, and Schroeter, 1983; Storrs *et al.*, 1989), others show a low incidence of sensitization. For example, in a study of 8149 patients in Europe, patch testing showed an incidence of only 0.21 per cent reaction to BNP (Frosch *et al.*, 1990). A similar low reactivity rate of 0.46 per cent in 2152 patients for patch testing to BNP was found in the United Kingdom (Shaw, 1997).

Toxicokinetics and metabolism

In vivo studies in the rat using ^{14}C-BNP showed that about 40 per cent of an epicutaneously applied dose was absorbed through skin in 24 h. Of the applied radioactivity, about 19 per cent was excreted in urine, faeces, and expired air. The 24 h recoveries of ^{14}C in urine and expired air were 15 per cent and 2 per cent, respectively, after the cutaneous application, compared with 74 per cent and 9 per cent for ^{14}C-BNP give intravenously. Urine TLC showed three metabolites, but no unchanged ^{14}C-BNP, following both epicutaneous and intravenous dosing (Buttar and Downie, 1980).

Ecotoxicology

BNP has a low volatility (vapour pressure 1.6×10^{-3} Pa). A $\log P_{ow}$ of 0.18 indicates a low bioaccumulation potential. BNP is hydrostable at pH 4 and 6. At pH 8 the $t_{1/2}$ is 2 months (Eriksson, Johnson, and Tornlund, 1995). Photolysis occurs at a high rate, with a $t_{1/2}$ of about 35 h. Biodegradation of 1.0 mg/L of ^{14}C-BNP at 22°C resulted in rapid transformation (78 per cent) after 3 days. Cited acute aquatic toxicity values are as follows:

Scenedesmus subspicatus	72-h EC_{50} = 0.02 mg/L; NOEC = 0.01 mg/L
Daphnia magna	48-h LC_{50} = 1.4 mg/L
Misido bahia	96-h LC_{50} = 5.9 mg/L
Brown shrimp	96-h LC_{50} = 121 mg/L
Oyster larvae	48-h EC_{50} = 0.69 mg/L (abnormal larvae)
	48-h LC_{50} = 1.7 mg/L

A reproduction study with *Daphnia magna* gave a 21-day NOEC of 0.27 mg/L. The Swedish National Chemicals Inspectorate has calculated a predicted no-effect concentration (PNEC) for aquatic ecosystems of 0.2 µg/L based on the lowest NOEC for algae.

iso-Thiazolones

Most commercially available *iso*-thiazolone biocides contain both 2-methyl-4-*iso*-thiazolin-3-one (MIT) and 5-chloro-2-methyl-4-*iso*-thiazolin-3-one (CMIT). Most tests for efficacy, mammalian toxicology, and ecotoxicology have been conducted using formulations containing both molecular species.

Identities and physicochemical properties

MIT

Structural formula: see Figure 9.5
CAS no.: 2682-20-4

Figure 9.5 2-methyl-4-isothiazolin-3-one. CAS no. 2682-20-4

Molecular weight: 115.16 g/mole
Melting point: 47–50°C
Boiling point: 93°C

CMIT

Structural formula: see Figure 9.6
CAS no.: 26172-55-4
Molecular weight: 149.60 g/mole
Melting point: 52–55°C
Sources of information: CIREP (1992); Lewis *et al.*, 1993

Mechanism of action

iso-Thiazolones are immediately bacteriostatic, but take several hours to achieve cidal activity. The sulphur atom of *N*-substituted *iso*-thiazolones is electrophilic and reacts with nucleophiles (Crow and Gorman, 1969). These electrophilic molecules target dehydrogenase enzymes in the Krebs cycle, affecting cellular metabolism (Chapman and Diehl, 1995). Cell damage can be measured by the loss of protein thiols. The following mechanism has been proposed:

- Covalent modification via direct electrophilic attack of thiols.
- Generation of a secondary electrophile by disulphide exchange and tautomerization to a thioacyl chloride.
- Intracellular generation of free radicals, stressing free radical defence mechanisms.

Figure 9.6 5-Chloro-2-methyl-4-isothiazolin-3-one. CAS no. 26172-55-4

Bacteria act as a sink for CMIT, concentrating 1 ppm as much as 400-fold, and leading to strong association of CMIT within the cell. As the concentration of CMIT increases, the rate of association and the rate of cidal effects increases (Diehl and Chapman, 1999).

Stability and decomposition

CMIT and MIT are less stable at alkaline pH, with hydrolysis beginning at pH 8. MIT is more stable at alkaline pH than CMIT. Cu^{2+} can be added as a stabilizer for CMIT, protecting it from nucleophilic attack. CMIT and MIT are also sensitive to thiols, reversing biostatic activity and preventing cell death (Chapman and Diehl, 1995).

Biocidal uses

MIT/CMIT have broad spectrum antimicrobial activity. The MIC for *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* have been determined to be 30 $\mu g/cm^3$ (Zeelie and McCarthy, 1983). Commercially available *iso*-thiazolones usually contain both CMIT and MIT in the ratio of 2.7:1, respectively. CMIT is the more effective biocide, being 100–1000-fold more active than MIT. CMIT is very water soluble and the mixture is commercially available in 1.5 to ≈14 per cent active solutions (Paulus, 1993, pp. 321–326). Other *iso*-thiazolones containing different alkyl chains or other modifications are available for particular applications.

iso-Thiazolones are used as preservatives in cosmetics and personal care. It is supplied to cosmetic manufacturers in the form of a mixture containing 0.35 per cent MIT and 1.15 per cent CMIT (1.5 MIT/CMIT; CIREP, 1992). They are also used in paints, slurries, adhesives, and other industrial applications at 5–15 ppm active. In pulp and paper applications, cooling towers, and recirculating systems, 0.5–1 ppm *iso*-thiazolone is added either continuously or as a fast feed slug dose. *iso*-Thiazolones sometimes are added in combination with, or alternated with, GA or DBNPA. To help prevent the development of bacterial tolerance, at least one preservative formulation is available in combination with BNP. *iso*-Thiazolones are also used to prevent marine biofouling of piers and boat hulls, and certain formulations are used in wood preservation. Details of the uses of MIT/CMIT in cosmetic applications and also noncosmetic applications are given in CIREP (1992).

Toxicology

Acute toxicology

Peroral. MIT/CMIT mixtures are moderately toxic to rats with LD_{50} values in the range of 40–79 mg ai/kg. A 1.5 per cent MIT/CMIT mixture had an LD_{50} of 3350 mg/kg. Signs of toxicity included those of severe gastric irritation, lethargy, and ataxia (CIREP, 1992). A 1.5 per cent solution of MIT/CMIT dosed in methylcellosolve to rabbits had an oral LD_{50} of 30 mg/kg. Signs of toxicity were

decreased motor activity, decreased breathing, and gastric irritation. *Intraperitoneal*. In aqueous solution, acute intraperitoneal LD_{50} values to Wistar rats were 4.6 mg/kg (males) and 4.3 mg/kg (females). The major sign was decreased motor activity and the principal lesion was peritonitis. *Percutaneous*. Several MIT/CMIT mixtures were tested with resultant LD_{50} values determined to be >75, 87, 94, 112, and 130 mg ai/kg.

Primary irritation

Skin. In New Zealand White rabbits and using standard occluded skin irritation tests and concentrations in the range 1.1–13.7 per cent, there was severe irritation. Aqueous dilutions of MIT/CMIT were tested on rabbit skin and 0.056 per cent ai was found to be non-irritating, 0.28 per cent ai was moderately irritating, 0.56 per cent ai was severely irritating, and 5.6 per cent ai was corrosive. In humans, the irritation potential of MIT/CMIT is concentration-dependant, with 400–800 ppm being strongly irritating, 200 ppm being slightly irritating, and 100 ppm essentially non-irritating (CIREP, 1992).

Eye. In rabbit eye irritation tests mixtures of MIT/ CMIT in the concentration range 1.1–14.0 per cent produced severe irritation, 0.56–1.7 per cent was moderately to severely irritating, 0.28 per cent slightly irritating, and 0.056 per cent essentially non-irritating (CIREP, 1992). In a cumulative rabbit eye irritation test, 0.1 ml of 0.0056 per cent MIT/CMIT was instilled into the conjunctival sac every 15 min for 2 h, and this procedure repeated daily for 5 consecutive days. Mild intermittent conjunctivitis, comparable to tap water controls, was seen. In a chorioallantoic membrane (CAM) test, 0.03 per cent MIT/ CMIT was non-irritating, but higher concentrations produced hyperaemia, haemorrhage, and coagulation (CIREP, 1992).

Sensitizing potential

Skin. In guinea pigs with up to 9 epicutaneous induction dosages applied over 3 weeks and a challenge dose 2 weeks later, delayed contact dermatitis developed, the incidence of which was dependant on both the induction and challenge concentrations. In one study the EC_{50} for guinea pigs challenged with 1000 ppm MIT/CMIT was 88 ppm (Chan *et al.*, 1983). The sensitizing potential of MIT/CMIT has been investigated extensively in humans (CIREP, 1992). There is general agreement that MIT/CMIT complex has sensitizing potential, although the potency of MCIT in this respect may be greater than that of MIT. However, there is some discrepancy in the cited eliciting concentration. Based on a wide ranging review of the extensive data, the Cosmetic Ingredients Review Expert Panel has concluded that for cosmetic preparations, MIT/CMIT may be safely used in 'rinse-off' products at a concentration not to exceed 15 ppm, and in 'leave-on' products at a concentration not to exceed 7.5 ppm; this conclusion refers to a mixture containing 23.3 per cent MIT and 76.7 per cent CMIT (CIREP, 1992).

Short-term repeated and subchronic toxicology

Inhalation. Male rats were exposed for 6 h/day, 5 days/week for 2 weeks to an aerosolized aqueous solution of MIT/CMIT at 0, 0.03, 0.07, and 0.13 mg ai/L. Mid- and high-dose rats had decreased body weight gain. There were a few deaths amongst low and high exposure concentration rats. Lesions included pulmonary haemorrhages and enlarged livers. Thus, under the conditions of this study the NOEC was <0.03 mg/L. *Peroral*. MIT/CMIT was included in the diets of rats for 3 months to give average daily dosages of 0, 3, 10, and 30 mg/kg. No mortalities occurred. There was a dosage-related increase in absolute and relative adrenal gland weights in females, and high dose males had slight but statistically significant increases in serum glutaric – oxaloacetic transaminase. There were no histopathological lesions. Beagle dogs had average daily dietary dosages of 0, 3, 10, and 30 mg/kg for 3 months. This resulted in no abnormal effects with respect to signs, chemical pathology, or histology. In another study rats received MIT/CMIT in the drinking water at average daily dosages of 0, 3, 8, and 20 mg/kg for 13 weeks. There was no mortality, but water consumption was decreased in all MIT/CMIT-treated rats. A significant decrease in serum globulin concentration with increased A/G ratio occurred in the high dosage females. Relative liver and kidney weights were increased in males and females of the high dosage group. Slight gastric irritation was seen in the high dose males and females. The NOAEL was $8\,mg\,kg^{-1}\,day^{-1}$, and the minimal effects dosage was $20\,mg\,kg^{-1}\,day^{-1}$. *Cutaneous*. Male and female New Zealand White rabbits had cutaneous applications of 0, 0.1, 0.2, and 0.4 mg/kg daily for 5 days/week for 13 weeks to both intact and abraded skin. Deaths, attributed to endemic respiratory disease, occurred in all treatment groups; 3/12, 5/12 and 4/12 respectively for the low, mid and high dosage groups, respectively. There was dosage-related slight to severe local erythema and slight oedema, but no treatment-related findings at necropsy or histology.

Chronic toxicology and oncogenicity

A chronic skin painting study was conducted in which 25 μl per rat of a distilled water solution containing 400 ppm MIT/CMIT was applied daily for 3 times/week for 30 months to the dorsal skin of male Charles River CD-1 mice. A positive control group received 1000 ppm 3-methylcholanthrene in acetone, and a negative control received tap water. In the MIT/CMIT group there was no indication of an increase in local or systemic neoplasams, but all animals of the 3-methylcholanthrene group had local squamous cell carcinomas within 6 months of the start of treatment.

Genetic toxicology

MIT/CMIT was mutagenic in *Salmonella typhimurim* strain TA 100 with and without metabolic activation, but not with strains TA98, TA1535, TA1537, or TA1538 (Wright *et al.*, 1983). When tested separately, CMIT but not MIT was

mutagenic in TA100 (Scribner *et al.*, 1983). MIT/CMIT was also mutagenic, without metabolic activation, in *Escherichia coli* WP2uvrA(p) (Wright *et al.*, 1983) and in mouse lymphoma L5178Y cells (CIREP, 1992). MIT/CMIT was not genotoxic in the following tests: unscheduled DNA synthesis using rat hepatocytes (Scribner *et al.*, 1983), *in vitro* chromosomal aberration test with Chinese Hamster lung fibroblasts, DNA binding *in vitro* with mouse lymphoma cell line, gender-linked recessive lethal test with *Drosophila melanogaster* (Scribner *et al.*, 1983), mouse *in vivo* cytogenetics assay, *in vivo* DNA binding with rat testis, and detection of induced cell transformation in mouse embryo fibroblast cell line C3H 10T1/2 (CIREP, 1992; Scribner *et al.*, 1983).

Developmental and reproductive toxicology

When given by gavage to Dutch belted rabbits over gestational days (gd) 6–18 at dosages of 0. 1.5, 4.4, and 13.3 mg ai kg^{-1} day^{-1}, MIT/CMIT was maternally toxic with dose-related mortalities and signs of toxicity (ataxia, diarrhoea, and gastric irritation). There were decreases in the number of live fetuses, increased resorption sites, and increased post-implantation losses, but no malformations. Thus, in this test, MIT/MIT was embryofetotoxic but not teratogenic at maternally toxic dosages (CIREP, 1992). When given by gavage to Sprague-Dawley rats over gd 5–15 at dosages of 0, 1.5, 4.5, and 15 mg kg^{-1} day^{-1} there was a low incidence of dosage-related mortalities and signs of toxicity, but no signs of embryofetotoxicity or teratogenic effects. Male and female Charles River rats were given MIT/CMIT in drinking water at daily average dosages of 0, 3, 8, and 20 mg/kg for 15 weeks. Rats from the same dosage groups were then mated. There were no adverse effects on fertility, reproduction, fetal survival, or fetal health (CIREP, 1992).

Toxicokinetics and metabolism

With the Sprague-Dawley rat, [^{14}C]MIT/CMIT was rapidly distributed following intravenous dosing with a total recovery of 94–111 per cent. A rapid elimination of ^{14}C from plasma with sustained maintenance of whole blood ^{14}C (3 μg/g) at 6–96 h post-dosing suggested ^{14}C to be erythrocyte-bound. In support of this was slow elimination of ^{14}C from spleen and liver. Tissue elimination was biphasic, with $t_{1/2}$ of >4 h. By 96 h faeces, urine, and $^{14}CO_2$ accounted for 35, 31, and 4 per cent of the administered dose, respectively. Following epicutaneous application, cutaneous recovery of [^{14}C]CMIT ranged from 89 to 94 per cent over a concentration range of 0.05–0.4 per cent, and was 13 per cent greater than [^{14}C]MIT at 0.2 per cent. Approximately 50 per cent of the radioactivity was associated with the treated skin. Elimination of ^{14}C from the application site had a $t_{1/2}$ of 13.1 days, suggesting a potential for local accumulation by repeated topical application. As the applied concentration was increased, the proportion of ^{14}C in skin decreased and that in excreta increased (Debethizy *et al.*, 1986). Other comparative studies in

the rat indicate that [^{14}C]MIT/CMIT were similar in the degree of absorption, binding, and excretory patterns following intravenous, peroral, and cutaneous dosing. However, MIT produced higher blood concentrations after oral and cutaneous dosing, and a 45 per cent greater relative absorption after peroral dosing than CMIT. Both dose-dependant and saturable processes governed absorption, distribution, and elimination of MIT/CMIT in the rat (CIREP, 1992).

Ecotoxicology

MIT/CMIT is completely soluble in water, and has relatively high vapour pressure at 8.3 Pa for MIT and 2.4 Pa for CMIT. Henry's constant is calculated to be 9.56×10^{-7} Pa $\cdot$ m^3/mole for MIT and 3.59×10^{-7} Pa $\cdot$ m^3/mole for CMIT, indicating low volatility. At pH 5 and 7, CMIT is hydrolytically stable, but at pH 9 hydrolysis occurs with a $t_{1/2}$ of 22 days. MIT is hydrolytically stable at pH 5, 7, and 9. An aerobic degradation study in river water using [^{14}C]CMIT resulted in conversion of 25 per cent to $^{14}CO_2$ after 29 days. Another aerobic degradation study with sediment/water at 25°C and [^{14}C]CMIT at a concentration of 1.0 mg ai/L resulted in rapid transformation with a DT_{50} of 17.3 h. After 7 days, ≈2.8 per cent of radioactivity had been evolved as $^{14}CO_2$ and 57 per cent was bound to sediment. An aerobic degradation study with [^{14}C]MIT in water/sediment at 25°C, and a test concentration of 1.0 mg ai/L, gave a DT_{50} of 9.1 h. At 7 days ≈9 per cent of radioactivity evolved as $^{14}CO_2$ and 68 per cent was bound to sediment. These studies indicate that MIT/CMIT is inherently, but not readily, biodegradable. MIT/CMIT have high water solubility and respective log P_{ow} values of −0.5 and 0.4, indicating a low potential for bioaccumulation. However, a bioaccumulation study in bluegill sunfish resulted in bioconcentration factors (BCFs) of 102, 114, and 67 for CMIT at respective doses of 0.02, 0.12, and 0.8 mg/L, suggesting that CMIT bioaccumulates. The BCF for MTI is 2.3 at a dose of 0.12 mg/L, indicating the absence of bioaccumulation. Acute toxicity values for MIT/CMIT are as follows:

Skeletonema costatum	72-h EC_{50} = 0.01 mg/L with NOEC < 0.01 mg/L
Daphnia magna	48-h EC_{50} = 0.18 mg/L
Brachydanio rerio	96-h LC_{50} <0.27 mg/L with NOEC = 0.084 mg/L
Cyprinodon variegates	96-h LC_{50} = 0.3 mg/L with NOEC = 0.18 mg/L

Prolonged testing indicates high toxicity:

Oncorrhyncus mykiss	24-h LC_{50} = 0.14 mg/L; 14-day LC_{50} = 0.07 mg/L; NOEC = 0.05 mg/L
Pimephales promelas	4-day LC_{100} = 0.23 mg/L; NOEC = 0.02 mg/L

A reproduction study with *Daphnia magna* resulted in a NOEC (21 days) of 0.1 mg/L.

Tetra-(hydroxymethyl)-phosphonium sulphate (THPS)

Identities and physicochemical properties

Structural formula: see Figure 9.7
CAS no.: 55566-30-8
Molecular weight: 406.49 g/mole
Boiling point: 108.5°C (75%)
Density: 1.3 g/ml (75%)
Appearance: clear straw-coloured liquid
Solubility: miscible with water
Sources of information: Paulus (1993, pp. 55–57, 137–138).

Mechanism of action

Although THPS is a quarternary phosphonium compound, its mode of action differs from that of other quarternary ammonium compounds. THPS has an acidic β-proton that is cleaved by base to form *tris*(hydroxymethyl)phosphine (THP) and formaldehyde (Hellman and Schumacher, 1960; Kirby and Warren, 1967). The phosphine is the biologically active species. It is known that Ph_3P reduces disulphides to thiols. THPS presumably acts as a reducing agent in a similar manner, disrupting the disulphide bonds in proteins and enzymes (Burns *et al.*, 1991; March, 1992; Overman and Petty, 1975; Overman, Smoot, and Overman, 1974). After reaction with disulphide, THPO is formed, which is no longer active (Overman and Petty, 1975).

Stability and deactivation

THPS is stable at acidic pH, but at alkaline pH it releases formaldehyde to form the active THP species. THP is unstable in the presence of disulphides, acting as a reducing agent to reduce disulphides and degrading to THPO. THPS is also unstable in the presence of oxidizers such as chlorine and peroxide. Both the oxidizer and THPS are consumed in these reactions. THPS is also inactivated by reducing agents such as bisulphite. If used in the presence of the oxygen scavenger,

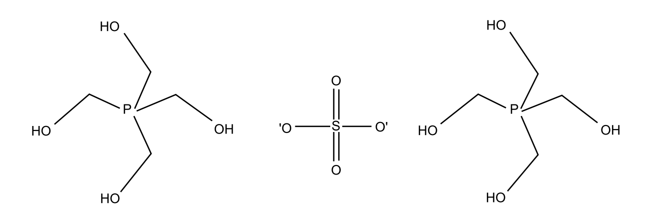


Figure 9.7 Tetrakis(hydroxymethyl) phosphonium sulphate. CAS no. 55566-30-8

ammonium bisulphite, THPS has been known to react with the bisulphite in water resulting in an increase in the dissolved oxygen content.

Biocidal uses

THPS is commercially available as an aqueous solution up to 75 per cent active. Some formulations contain surfactants. THPS is a fast acting biocide with activity against Gram-positive and Gram-negative bacteria, fungi, and algae as well as sulphate-reducing bacteria. THPS can be added continuously or as a slug dose. It is used as an oilfield biocide at 10–300 ppm in slug doses. It is also used in pulp and paper manufacturing (15–350 ppm), although not in conjunction with oxidizing biocides. THPS has some non-biocidal uses such as leather tanning and as a FeS scavenger.

Toxicology

Chronic toxicity and oncogenesis

F344/N rats and $B6C3F_1$ mice received 0, 5, or 10 mg THPS/kg in distilled water by gavage daily for 5 days/week for 103 or 104 weeks. Mean body weights of rats and mice were comparable with those of controls and no signs of toxicity were seen. There was a dosage-related increase in the incidence of cytoplasm vacuolization of liver cells in rats, of focal hyperplasia of the adrenal medulla in high dose male mice, and of thyroid gland follicular cell hyperplasia in high dosage female mice. There was no evidence for carcinogenicity of THPS in either sex of rats or mice (NTP, 1987).

Genetic toxicology

THPS was not active in a *Salmonella typhimurium* reverse mutation test with and without metabolic activation (Ulsamer, Osterberg, and McLaughlin, 1980). THPS induced forward mutations in mouse lymphoma L5178Y cells (NTP, 1987). In a micronucleus test THPS produced small increases in micronuclei at 700 and 1000 mg/kg. In a dominant lethal assay using ICR mice, THPS produced a significant decrease in the number of pregnant females and an increase in the number of mortalities at 1000 mg/kg. There was no increase in the average number of implants per pregnancy (Ulsamer, Osterberg, and McLaughlin, 1980).

Peracetic acid (PAA)

Identities and physicochemical properties

Structural formula: $H_3C—CO—O—OH$
CAS no.: 79-21-0
Synonyms: peroxyacetic acid; acetyl hydroperoxide; PAA
Molecular weight: 76.05 g/mole
Freezing point: −30°C

Boiling point: 105°C (40% solution)
Specific gravity: 1.15 (20°C)
Flash point: 56°C
Appearance: clear liquid
Odour: acrid type
Sources of information: Lewis (1993); NAS (1995); Paulus (1993)

Mechanism of action

PAA acts as a protein denaturant and oxidatively disrupts sulphhydryl and sulphur bonds within essential metabolic enzymes (Fraser, 1986; Lefevre, Audic, and Ferrand, 1992). In addition, PAA disrupts membrane transport by rupturing or dislocating cell membranes, and thereby altering the chemiosmotic function (Fraser, 1986). The mechanism for this disruption is likely by radical formation.

Stability and decomposition

Lower concentrations of PAA are in equilibrium with acetic acid, peroxide, and sulphuric acid. The sulphuric acid is added to favour an equilibrium towards PAA. Eventually the PAA decomposes to acetic acid and water (Block, 2001; Fraser, 1986). Concentrated PAA is not very stable unless stabilizers are added to prevent decomposition to acetic acid and water. PAA is less active in the presence of organics, and most stable at acidic pH. PAA is most active at pH 2.5–4.0, and inactivated by thiols and other reducing agents (Block, 2001; Paulus, 1993, pp. 430–432). Concentrated PAA requires to be handled with care because of the potential to be explosive at relatively low temperatures (Paulus, 1993, pp. 430–432).

Biocidal uses

PAA is commercially available as a general biocide as an equilibrium mixture with acetic acid, hydrogen peroxide, and sulphuric acid in water at about 50 per cent PAA. It is also available as an ≈35 per cent concentrate for instrument sterilization. PAA is regarded as a highly effective disinfectant, but experience with the substance is limited and it is suggested that it should be used in sealed or exhaust ventilated facilities (Working Party, 1998). It is more expensive than GA, less stable, and larger volumes have to be stored. In a comparative study with chlorine dioxide, PAA was found to produce a high abatement of microorganisms and was considered to be a valuable alternative in the disinfection of waste water (Stampi *et al.*, 2002).

PAA is considered more potent than peroxide and better able to control bacteria and spores at safe levels (Block, 2001). PAA has broad spectrum activity. Aside from being active against bacteria, fungi, and viruses, it is also mycobacteriocidal and sporicidal. Therefore, PAA is one of the few biocides capable of low-temperature liquid high-level

disinfection and sterilization (0.1–0.5 per cent active). In addition, PAA vapour can also be used as a sterilant/high-level disinfectant. One commercially available sterilant uses concentrated PAA that is diluted to ≈0.2 per cent active at 55°C in a specially designed reprocessor (Schneider, 1994). This is a single-use process, requiring fresh PAA for each cycle. Another manufacturer makes a sterilant/high-level disinfectant at a much lower concentration (0.08 per cent). This formulation also contains 1 per cent hydrogen peroxide and is reusable up to 14 days. Since PAA degrades into acetic acid and water, it is regarded as easy to use and to dispose of. It is, however, somewhat corrosive and repeated use as a sterilant requires the use of a corrosion inhibitor to preserve the integrity of the instruments (Schneider, 1994). PAA is used to disinfect food processing equipment including meat processing, dairies, and breweries. Because of the fast decomposition of PAA, these surfaces usually do not require rinsing after use. The low concentration equilibrium formulations are used to treat cooling towers and water recirculating systems in slug doses of 1–10 ppm. PAA is also one of the few biocides used to reprocess renal dialysers and to control mould growth on fruits and vegetables. It has also been convenient and effective as a sterilant for apparatus and equipment used to maintain gnotobiotic animals (Fordham, 1978).

Toxicology

Acute toxicity

Peroral. The rat peroral LD_{50} is 1540 mg/kg for a 40 per cent solution (Anonymous, 1982; NAS, 1995). *Percutaneous.* The LD_{50} for the rabbit is 1410 mg/kg (NAS, 1995).

Primary irritancy

Skin. A 40 per cent solution is corrosive (NAS, 1995). *Eye.* At 0.1 per cent in water, PAA is not irritating to the rabbit eye. A 10 per cent solution causes ulceration and perforation of the cornea and formation of synblepharon (Grant and Schuman, 1993). A 40 per cent solution can cause severe eye burns (NAS, 1995).

Peripheral sensory irritation

PAA is a relatively potent peripheral sensory irritant. In mice, a 1-h RD_{50} of 5.4 ppm has been determined (Gagnaire *et al.*, 2002). The authors suggest that this value indicates a TLV-STEL of 0.5 ppm (0.1 RD_{50}) and a TLV-TWA of 0.2 ppm (0.03RD_{50}).

Genetic toxicology

When given by intraperitoneal injection at a dosage of 25 mg kg^{-1} day^{-1} for 35 days, PAA produced a slight increase in the rate of mouse sperm head abnormalities (Koch *et al.*, 1989). PAA did not induce DNA repair synthesis (Coppinger, Wong, and Thompson, 1983).

Occupational medical aspects

Skin or eye contact with a 40 per cent solution will cause serious burns. Inhalation of high vapour concentrations or mists will lead to burning sensations in the nose, throat, and chest, and coughing, wheezing, and shortness of breath (NAS, 1995).

Glutaraldehyde (GA)

Identities and physicochemical properties

Molecular formula: $OHC \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CHO$
CAS no.: 111-30-8
Synonyms: glutaral; glutaric dialdehyde
Molecular weight: 100.13
Freezing point: −21°C (50%)
Boiling point : 100.5°C (50%) (760 mm Hg)
Density (20°C): 50% = 1.129 g/ml
25% = 1.064 g/ml
10% = 1.026 g/ml
2% = 1.006 g/ml
Vapour pressure (25°C): 50% = 0.116 mm Hg
15% = 0.041 mm Hg
2% = 0.0051 mm Hg
Vapour concentration (25°C): 50% = 207 ppm
15% = 53 ppm
2% = 7 ppm
0.1% = 0.3 ppm
0.01% = 0.03 ppm
Henry's Law constant: 0.000033 L atm/mole
Odour: apple-like
Odour threshold: 0.04 ppm
Solubility (20°C): miscible with water, acetone and *iso*propanol
methylene chloride = 36 g/L
ethyl acetate = 30 g/L
toluene = 4.4 g/L
n-hexane = 0.96 g/L
$P_{ow} = 0.98$
Sources of information: BIBRA (1991); CIREP (1996); Olson (1998)

Mechanism of action

GA is an aliphatic dialdehyde which is capable of reacting with amines and thiols. When GA reacts with amines, it forms a stable six-membered cyclic compound that can be further reacted with another GA molecule or nucleophile (Banin *et al.*, 1992;

Berrien, Royer, and Husson, 1994; Francois *et al.*, 1998). GA has been used to synthesize cyclic organic compounds in this manner (Banin *et al.*, 1992; Berrien, Royer, and Husson, 1994). GA reacts with amino acids (such as the free amine of lysine) in proteins in a similar manner, producing a cross-linked cell. The formation of a stable six-membered ring is likely the reason GA is a potent biochemical dialdehyde. In addition to cross-linking, there are indications that GA inhibits protein synthesis precursor uptake and some cellular enzymes (Scott and Gorman, 2001). The non-specific nature of the mechanism of action of GA leads to broad spectrum activity. GA biocidal activity increases at alkaline pH, presumably because as the pH reaches the pK_a of the available amines, the unprotonated amines become more susceptible to reaction with GA.

Stability and decomposition

GA is stable at pH 3.0–4.5. As the pH increases (>10) GA forms both soluble and insoluble polymers through aldol condensation. GA is inactivated by bisulphite and primary amines. Complex formation with bisulphite is reversible at elevated temperature (>55°C). GA is manufactured commercially as a 50 per cent aqueous solution because 100 per cent GA is very unstable and forms clear glass polymers in a relatively short time.

Biocidal uses

GA is commercially available in aqueous solutions (≈20–50 per cent) (Paulus, 1993, pp. 37–38, 45–47). There are some formulations with ADAC quats at a ratio of 7:1, (e.g. GA:ADAC). Instrument sterilant formulations are typically 2–4 per cent active GA, some containing surfactants.

GA is used as a general biocide at 25–200 ppm active in pulp and paper processing, cooling water towers, water recirculating systems, and oil field recovery (Paulus, 1993, pp. 37–38, 45–47; Scott and Gorman, 2001). GA is also used in some industrial preservative applications at 100–1000 ppm active, and as an animal housing sanitizer (1000 ppm). It also finds use in reprocessing renal dialysers. GA is sometimes used in conjunction with other biocides such as quarternary ammonium compounds. GA is a fast acting biocide, effective against Gram-positive and Gram-negative bacteria, yeast, fungi (>500 ppm), and sulphate-reducing bacteria. GA has been shown to be effective against biofilm. Any reduced activity is linked to transport limitation rather than phenotypic change in the biofilm (Grobe and Stewart, 2000). Since GA is effective against mycobacteria and spores, it is one of the few biocides available that can be used as a cold high-level disinfectant or sterilant for endoscopes and other thermosensitive devices (Scott and Gorman, 2001). When used as a sterilant, most acidic solutions of GA (≈2.5 per cent active) are activated by a buffer system to produce an alkaline solution (Ballantyne, Myers, and Blasczak, 1997). These sterilants are reusable for up to 28 days. They can be

used for manual disinfection as well as in automatic reprocessors. The instruments require at least a 20-min soak in the sterilant at 20°C and then copious rinsing to remove residual GA from the endoscope (Ballantyne and Jordan, 2001). Unlike many other biocides, GA also has many non-biocidal uses, including leather tanning, tissues fixation, enzyme immobilization, embalming, and cross-linking bioprostheses such as heart valves.

Toxicology

Acute toxicity

Peroral. Aqueous solutions of 5 per cent and higher are of moderate acute peroral toxicity, and those of 2 per cent and lower are of slight toxicity, with the mouse being more sensitive than the rat (Ballantyne and Myers, 2001). Typical LD_{50} values are given in Table 9.1. Expressed as the volume of solution dose (ml/kg), the LD_{50} values increase with dilution, but when expressed as the amount of GA given (mg GA/kg) the values decrease with increasing dilution. This may have implications for the first-aid management of swallowed GA solutions. Mortality usually occurs within 1–3 days. Dilute solutions produce only minor irritation of the upper alimentary tract, but more concentrated solutions produce severe irritation and corrosive lesions of the upper alimentary tract. Signs of toxicity include piloerection, sluggishness, rapid breathing, and diarrhoea. Survivors recover from these effects by 1–5 days after dosing, and generally do not show any gross pathology. Decedants usually show congestion and

Table 9.1 Acute lethal peroral toxicity of aqueous solutions of various concentrations of glutaraldehyde for male rats and mice[a]

Species	GA (% w/w)	LD_{50} (95% confidence limits)	
		ml/kg[b]	mgGA/kg[c]
Rat	50	1.30 (0.27–1.94)	733 (452–1095)
	45	1.19 (0.84–1.69)	538 (422–849)
	25	1.54 (1.14–2.08)	410 (303–553)
	15	1.17 (0.72–1.88)	183 (112–293)
	10	1.62 (1.01–2.62)	166 (104–269)
	5	3.25 (2.40–4.39)	170 (122–222)
	2	3.34 (2.53–4.43)	67 (51–89)
	1	12.30 (9.13–16.70)	123 (92–168)
Mouse	50	0.27 (0.18–0.40)	152 (102–225)
	25	0.65 (0.32–1.30)	173 (85–346)
	5	0.62 (0.36–1.04)	31 (18–53)
	1	3.36 (1.35–8.41)	34 (14–84)

[a]Data from Ballantyne and Myers (2001).
[b]As volume of glutaraldehyde solution dosed.
[c]As contained glutaraldehyde dosed.

Table 9.2 Acute percutaneous lethal toxicity of various concentrations of aqueous glutaraldehyde for male rabbits[a]

GA (% w/w)	LD_{50} (95% confidence limits)	
	ml/kg[b]	mg GA/kg[c]
50	2.54 (1.46–4.41)	1434 (824–2489)
46	2.00 (1.18–3.39)	1004 (593–1703)
25	8.80 (1.91–33.5)	2341 (508–8911)
15	killed 0/5	

[a]24-h occluded contact and 14-day observation period. Data from Ballantyne and Myers (2001).
[b]As volume of glutaraldehyde solution dosed.
[c]As contained glutaraldehyde dosed.

haemorrhage of the gastric mucosa with thickening of the pyloric area, and congestion of the small intestinal mucosa.

Percutaneous. Aqueous solutions of 45 per cent and higher are of moderate acute percutaneous toxicity, those of 25 per cent are of slight toxicity, and 15 per cent solutions do not produce systemic toxicity (Table 9.2; Ballantyne and Myers, 2001).

Vapour exposure. Exposure to vapour generated dynamically or statically at ambient temperature does not produce mortalities, and signs are limited to those of respiratory tract and ocular irritation (blepharospasm, lacrimation, nasal discharge, and abdominal and mouth breathing). Typical exposure situations for ambient generation conditions are shown in Table 9.3. Definitive toxicity is seen only in animals acutely exposed to GA vapour generated at high temperature (Ballantyne

Table 9.3 Acute vapour exposure studies conducted at ambient temperature with rats, and vapour generated to give saturated vapour conditions[a]

Generation	GA vapour (ppm)[b]	Exposure time (h)	Mortality[c]	Signs
Static	48.1 ± 6.77	6	0/6	Lacrimation Perinasal discharge
	4.9 ± 3.4	6	0/6	Periocular wetness Labored breathing
Dynamic	8.1 ± 2.2	4	0/10	Blepharospasm Rhinorrhea
	16.3 ± 15.0	4	0/10	Blepharospasm Audible breathing
	14.5 ± 2.1	4	0/10	Blepharospasm Periocular wetness

[a]Data from Ballantyne and Myers (2001).
[b]As mean ± SD.
[c]As number dying/number exposed.

and Myers, 2001). For example, when rats were exposed to GA vapour generated at 65°C, they showed an exposure–concentration-related mortality pattern, allowing the calculation of 4-h LC_{50} values of 23.5 ppm for males, and 40.1 ppm for females. A further study compared the acute vapour exposure toxicity to rats of GA vapour generated at ambient (24°C) versus elevated (60°C) temperature (Ballantyne and Myers, 2001). Under elevated temperature conditions there were 4-h LC_{50} values of 36.9 ppm for males and 44.3 ppm for females. Signs included blepharospasm, lacrimation, mouth and audible breathing, and hypoactivity. In contrast, with ambient temperature conditions, and GA vapour concentrations of 27.1 and 27.4 ppm, there were no mortalities. The marked differences between the elevated and ambient temperature generation findings may, in part, be due to the ability to generate higher vapour concentrations at the elevated temperature, but the possibility that a toxic species is produced during the heating of GA solutions cannot be excluded. For example, in a 4-h saturated vapour study (23°C) in which the GA vapour concentration was 22.2 ppm there were no mortalities. In contrast, in a study with vapour generation at 65°C and a vapour concentration of 23 ppm 2/6 males and 2/6 females died. These findings, at comparable GA vapour concentrations, suggest the generation of a toxic species under the elevated temperature conditions.

Primary irritation

Skin. Standard rabbit primary skin irritation studies (4-h occluded contact with 0.5 ml) have shown concentration-related effects (Table 9.4). At 45–50 per cent, aqueous solutions of GA produce severe local inflammation (erythema, oedema, desquamation) and corrosion; at 25 per cent there is moderate inflammation; at 5–10 per cent minor to moderate inflammation; and 1 per cent is threshold. Alkalinization of 2.2 per cent aqueous GA solutions has no significant effect on skin irritating potential (Ballantyne, Myers, and Blasczak, 1997). In a controlled study

Table 9.4 Primary irritant effects of 0.5 ml of various concentrations of glutaraldehyde in water applied under occluded conditions to shaven dorsal trunk skin of rabbits[a]

GA (% w/w)	Contact time	Observations
50	4 h	Necrosis
50	1 h	Erythema, oedema, desquamation, alopecia, necrosis
50	3 min	Minor transient erythema
45	4 h	Moderate erythema, mild oedema, punctate necrosis
25	4 h	Moderate erythema, mild oedema, punctate necrosis
10	4 h	Moderate erythema, mild oedema, punctate necrosis
5	4 h	Minor erythema and oedema
2	4 h	Minor erythema
1	4 h	None

[a]From Ballantyne and Myers (2001).

with human volunteer subjects it was found that 0.5 per cent aqueous GA solution was slightly irritant (erythema), 0.2 per cent was marginal, and 0.1 per cent was not irritant (Ballantyne and Berman, 1984). Regional variations in cutaneous irritant effects occur, which are primarily related to the thickness of the skin (Juhlin and Hannson, 1968; Prigant, Ibbora, and Merley, 1996; Reifenrath *et al.*, 1985).

Eye. Rabbit eye irritation tests (Ballantyne and Myers, 2001) have shown that the lowest concentration of GA to produce corneal injury is 1 per cent, and the no-effects concentration is 0.5 per cent. Corneal injury is mild at 2 per cent GA, but severe at 5 per cent. For conjunctival irritation, the threshold is 0.2 per cent GA and the no-effects concentration is 0.1 per cent. At 1–2 per cent, conjunctival hyperaemia and chemosis are marked. Alkalinization of 2.2 per cent GA solution with buffer resulted corneal injury and conjunctival inflammation being more and persisting longer than with acidic solutions (Ballantyne, Myers, and Blasczak, 1997). Only a few case reports of human eye injury from GA have been published (Dailey, Parner, and Aminlari, 1993; Murray and Ruddy, 1985).

Sensitizing potential

Skin sensitization. Evidence for a low incidence of skin sensitization comes from laboratory animal studies, controlled human volunteer studies, and clinical and occupational case reports. A contact hypersensitivity-type reaction has been demonstrated with the mouse ear swelling test (MEST) (Descotes, 1988; Gad, Dunn, and Dobbs, 1986; Sailstad, Tepper, and Doerflex, 1993). Mouse local lymph node proliferate assays (LLNAs) have shown concentration-related stimulation of nodal activity, indicating a skin sensitizing potential for GA (Ballantyne and Jordan, 2001; Dearman *et al.*, 1999; Hilton *et al.*, 1998). A dosage-related hypersensitivity response was obtained in the guinea pig and mouse that was statistically significant at 0.3 per cent in mice and 3 per cent in both species (Stein *et al.*, 1987). Unbuffered 2.2 per cent GA had a greater skin sensitizing potential than buffered GA (pH 7.8) in a guinea pig maximization study (Ballantyne, Myers, and Blasczak, 1997). In a repeated insult patch test with human volunteer subjects (Ballantyne and Berman, 1984) it was found that 0.1 per cent and 0.2 per cent GA did not cause a sensitizing reaction, but with 0.5 per cent GA induction there was a challenge reaction in one of 109 subjects. There are several reports of allergic contact dermatitis from occupational exposure to GA and also cases of skin sensitizing reactions to GA when used in the treatment of various dermatological diseases (reviewed by Ballantyne and Jordan, 2001). There is some evidence for regional variations in susceptibility to skin sensitization with GA, and which appear to be related principally to differences in skin thickness (Maibach and Prystowsky, 1977). Cross-sensitization to formaldehyde does not occur (Gordon, 1983; Lyon, 1971; Maibach, 1975), but cross-sensitization to other dialdehydes is possible (Zemtsov, 1992). The available evidence indicates that GA has a potential to cause skin sensitization, with an eliciting threshold concentration of 0.5 per cent. Alkalinization

of GA solutions may reduce the incidence of skin sensitizing reactions (Ballantyne, Myers, and Blasczak, 1997). Rarely, allergic contact dermatitis has resulted from exposure to GA vapour (Fowler, 1989). GA does not produce phototoxic or photoallergic reactions (Ballantyne and Jordan, 2001).

Respiratory sensitizing potential. A guinea pig bronchial hypersensitivity study did not show any change in breathing rate, or respiratory waveform after GA vapour challenge exposures (Werley, Burleigh-Flayer, and Ballantyne, 1995). However, a murine IgE induction study produced a concentration-related increase in serum IgE that was significant for 25 per cent and 10 per cent GA, but not 5 per cent GA (Ballantyne and Jordan, 2001). Also, in a cytokine secretion profile study in BALB/c mice (Dearman *et al.*, 1999), high levels of interleukin-4 and -10, but low levels of interferon-γ were produced, which is typical of a TH2 response. Several publications have drawn attention to the possibility that asthmatic-like symptoms may occur following exposure to GA vapour in the occupational environment (summarized by Ballantyne and Jordan, 2001). Some patients have been investigated with respect to the possible role of the immunological system (Curran, Burger, and Wiley, 1996; DiStefano *et al.*, 1998, 1999). The correlation between specific IgE antibodies and clinical symptoms is poor, and the role of IgE in the pathogenesis of GA occupational asthma is currently unclear. In most cases the clinical findings are similar to those of the reactive airways dysfunction syndrome (RADS), and result from overexposure to GA vapour. In situations where there are good industrial hygiene controls on potential exposure, then occupational asthma does not occur. Thus, in an epidemiological study involving 218 workers assigned to GA production or drumming units, there was no indication of skin or respiratory sensitizations related to GA exposure (Teta *et al.*, 1995). A cross-sectional study of 135 nurses using GA solutions for instrument cold sterilization was conducted in 26 hospitals in Australia (Pisannello *et al.*, 1997). There was no evidence for an increased incidence of respiratory effects, including asthmatic symptoms, in the nurses exposed to GA. A large cross-sectional survey of endoscopy nurses was conducted in the United Kingdom, using 348 currently employed nurses (Vyas *et al.*, 2000). Whilst nurses exhibited irritant symptoms, there were neither clinical nor investigational (respiratory function tests, specific IgE antibody studies) indications of asthma and no evidence that GA is a respiratory sensitizer.

Peripheral sensory irritation

GA is a typical peripheral sensory irritant (PSI) material, and can interact with sensory nerve receptors in the skin and exposed mucosal surfaces, resulting in local sensations and related reflexes (Ballantyne, 1999). Exposure to suprathreshold concentrations of GA vapour may result in eye discomfort, excess lacrimation, discomfort in the nose and chest, rhinorhea, cough, and sneezing. The PSI effects of GA have been investigated by an animal model and by studies with human volunteer subjects. Measurement of the depression of respiratory rate in ND4 Swiss Webster mice gave a calculated RD_{50} of 13.86 ppm (Werley *et al.*, 1995). Using a

correlative correction factor of 0.03 (Alarie, 1984), it was calculated that 0.2 ppm is a value at which sensory irritation would be anticipated to be negligible in humans. A lower RD_{50} value of 2.6 ppm has been reported for Swiss OF1 mice (Zissu, Gagnaire, and Bonnet, 1994). One human study demonstrated a sensory irritant threshold for GA vapour of 0.24–0.26 ppm (Whitmore, 1976), and another study gave a human irritation threshold of 0.3 ppm (Colwell, 1976).

Subchronic repeated exposures

Peroral. Because the irritant effects of GA may limit dosing by the epicutaneous and inhalation routes of exposure, studies have been conducted by the peroral route to permit maximum dosage, and hence, allow the greatest potential for the expression of toxicity. Drinking water studies have been conducted in the rat, mouse, and dog, with dosing up to 3 months and full monitoring for signs, body weight, food and water consumption, haematology, clinical chemistry, urinalysis, necropsy, organ weights, and histology. Dosages were up to 1000 ppm for rats and mice and 250 ppm for dogs. The major, and only, findings in all species were decreased food and water consumption (probably related to an aversion to the taste and/or irritancy of GA), decreased body weight, and decreased urine volume with increased specific gravity. In no species was there any clinical, haematological, biochemical, or morphological evidence for target organ or tissue systemic toxicity. All the findings are compatible with decreased urine production secondary to decreased water consumption resulting from an aversion to GA in the drinking water (Hermansky, Ballantyne, and Fowler, 1995a).

Epicutaneous. Fischer 344 rats had 20 epicutaneous occluded applications (6 h/day) of aqueous GA solutions resulting in dosages of 50, 100, and 250 mg kg^{-1} day^{-1} (Werley *et al.*, 1996). There were no treatment-related mortalities or signs of systemic toxicity. Local skin irritation was minimal (minor erythema and slight oedema). There were slight decreases in body weight (at 250 mg kg^{-1} day^{-1}), reduced body weight gain (100 and 250 mg kg^{-1} day^{-1}), and slightly reduced food and water consumption (250 mg kg^{-1} day^{-1}). The only haematological findings (females only) were increased platelet and reticulocyte counts. Clinical chemistry results showed a slight increase in blood urea nitrogen. Urinalysis was normal. Adrenal gland weights were slightly increased for the 100 and 150 mg kg^{-1} day^{-1} females. Histopathological findings were only seen in the dosed skin area, and consisted of acanthosis, hyperkeratosis, parakeratosis, dermatitis, epidermitis, and dermal fibrosis. The minor effects noted are common findings in rodents receiving cutaneous applications of irritant materials (Hermansky *et al.*, 1995b). There was no evidence for systemic target organ or tissue toxicity.

Vapour exposure. Several short-term (9–12 days) repeated vapour exposure studies have been conducted in rats and mice as preliminary investigations to definitive subchronic studies. These have been reviewed by Ballantyne and Jordan (2001). Although conducted under differing conditions of vapour generation, vapour analysis, and monitoring, the following are common findings. For vapour concentrations of 2 ppm and

greater, mortality was exposure–concentration-related. The lowest concentration of GA vapour causing mortality was 0.63 ppm (1/20 rats), and all studies showed a steep slope on the vapour-concentration–mortality data. The available evidence suggests a greater degree of lethality for vapour generated at elevated temperature (*c*. 50°C) than for vapour generated at ambient temperature. All studies showed effects on the respiratory tract typical of those for an irritant material. At ≥ 5 ppm, respiratory tract histopathology was extensive, but at ≤ 1.6 ppm effects were generally restricted to the nasal mucosa. Threshold concentrations for effects in the nasal mucosa were 0.3 ppm (rats) and 0.5 ppm (mice), and the no-effects concentration was 0.16 ppm for both species. A detailed subchronic vapour exposure study was conducted with Fischer 344 rats at concentrations of 20, 50, and 200 ppb, with exposures for 6 h/day, 5 days/week, for a total of 14 weeks. There were minimal signs of respiratory tract and ocular irritation, and slightly decreased body weight, at 50 and 200 ppb, but no evidence for respiratory tract inflammation or systemic toxicity (Greenspan *et al.*, 1985). In another study, $B6C3F_1$ mice were exposed to 100 ppb GA vapour for 6 h/day, 5 days/week for 52 or 78 weeks. Lowered body weight gain was measured for female mice, but males gained weight. Histopathological effects seen in female, but not male, mice and were restricted to the nasal vestibule. The findings included hyperplasia of the squamous epithelium lining the dorsal wall and lateral aspect of the atrioturbinate, and was associated with intra-epithelial and subepithelial cellular infiltration (granulocytes and lymphocytes). This lesion was significantly increased ($P < 0.05$) in female mice of the 52-week (15/48 v. 2/49 controls) and 78-week (14/28 v. 6/28 controls) exposure groups. Additionally, epidermal ulceration and erosions were seen with squamous cell and inflammatory exfoliation in the nasal cavity, but there was no squamous metaplasia. Also, there was no histological evidence for systemic toxicity (Zissu, Bonnet, and Binet, 1998). In a more extensive study conducted under the US National Toxicology Program, Fischer 344 rats and $B6C3F_1$ mice were exposed for 6.5 h/day, 5 days/week, for 13 weeks, to GA vapour concentrations of 0, 62.5, 125, 250, 500, and 1000 ppb. Monitors for toxicity included signs, body weight, haematology, clinical chemistry, sperm morphology, vaginal cytology, and gross and microscopic pathology. The only sign with rats was difficulty with breathing (1000 ppb). Mice were more susceptible, with 10 per cent mortality at 500 ppb and all animals dying at 1000 ppb. Histopathology was restricted to the nasal mucosa and (mice only) larynx, and consisted of inflammation, hyperplasia, and squamous metaplasia. Effects were moderate at 1000 ppm and mild at ≤ 500 ppb. A no-effects concentration for nasal mucosal effects from GA vapour was established at 62.5 ppb for the male rat. Minimal inflammation was present in half of the female mice at 62.5 ppb (NTP, 1993b).

Chronic toxicity and oncogenicity

Studies have been conducted by the peroral and inhalation routes. The peroral study was conducted by lifetime (2-year) exposure of Fischer 344 rats to GA in the drinking water at concentrations of 50, 250, and 1000 ppm, resulting in respective average daily

dosages of 3.6, 17.1, and 63.9 mg/kg for male rats and 5.5, 25.1, and 85.9 mg/kg for females (Van Miller *et al.*, 2002). Interim sacrifices were conducted at 12 and 18 months after the start of dosing. No dosage-related mortality occurred. Absolute body weights and body weight gains of the 250 and 1000 ppm males and females were reduced over the study in a dosage-related manner. Food and water consumption by the 250 and 1000 ppm groups were decreased in a statistically significant dose-related manner over the study, and mean water consumption by the 50 ppm rats was slightly reduced but not with statistical significance. The 250 and 1000 ppm groups had a dosage-related decrease in urine volume with increased osmolality and slightly reduced pH. Absolute kidney weights were increased in the 250 and 1000 ppm groups at the 12 and 18 months sacrifices, and decreased at the 2-year sacrifice. Relative kidney weights were increased at all sacrifice times for the 1000 ppm group, at 52 weeks for the 250 ppm group, and at 72 weeks for the 50 ppm group. The urinalysis and renal weight changes are compatible with a physiological compensatory adaptation to reduced water consumption. Gross and histological evidence of gastric irritation was observed principally in the 1000 ppm rats euthanized at 2 years and in animals dying during the study. Bone marrow hyperplasia and renal tubular pigmentation, seen in rats that died and at the 2-year sacrifice, may have been secondary to a low grade haemolytic anaemia in animals with large granular lymphocytic leukaemia (LGLL; Stromberg *et al.*, 1983). The only neoplasm that showed a statistically significant increase was LGLL, which occurred at a high incidence in both sexes and in all groups, including controls, for both animals that died and at the 2-year sacrifice. The overall incidences for LGLL in the spleen of the 0, 50, 250, and 1000 ppm groups were, respectively, 43, 51, 40, and 46 per cent for males, and 24, 41, 41, and 53 per cent for females. In assessing the significance of the increased LGLL it needs to be noted that the increased incidence occurred only in females, that LGLL is a spontaneously occurring neoplasm in the Fischer 344 rat (Losco and Ward, 1984; Hermansky, Longhorn, and Ballantyne, 1992), and that the incidence of LGLL in control Fischer 344 rats shows temporal intra- and inter-laboratory variations (Hermansky, Longhorn, and Ballantyne, 1992; Stefanski, Elwell, and Stromberg, 1990). It is possible that the low incidence of LGLL in the control female rats was a consequence of random biological variation. However, even if the increase in LGLL was treatment-related, it is unlikely to have been the result of a genotoxic carcinogenesis. No clear dosage–response relationship was apparent, and there was no increased incidence in males. Also, genetic toxicology studies have failed to demonstrate a mutagenic or clastogenic effect in the intact organism (see below). There is the possibility that the increased incidence of LGLL in female Fischer 344 rats was a consequence of the chronic dosing with GA having a modifying effect on one or more of the factors influencing the expression of this spontaneously occurring neoplasm in the female rat.

A 2-year chronic toxicity and oncogenicity study was conducted with GA vapour by the inhalation route with Fischer 344 rats and $B6C3F_1$ mice (Van Birgulen *et al.*, 2000). For rats, groups of 50 males and 50 females rats were exposed to 0, 250, and 750 ppb GA vapour for 6 h 25 min/day, 5 days a week for 104 weeks. The 2-year survivals for

the 0, 250, 500, and 750 ppb groups were, respectively, 12/50, 14/50, 6/50, and 6/50 for males and 26/50, 3/50, 15/50, and 14/50 for females. Mean body weights of all males and the 500 and 750 ppb females were generally slightly reduced compared with the 0 ppb controls. Histopathological effects were mainly limited to the nasal mucosa and seen as exposure–concentration-related squamous epithelial hyperplasia, squamous metaplasia, inflammation, goblet cell hyperplasia, and olfactory epithelial hyaline degeneration. Inflammation consisted of infiltrates of neutrophils, lymphocytes, and plasma cells. Squamous epithelial hyperplasic and inflammation were present at 250 ppb with statistical significance. Similar sized groups of mice were exposed to 0, 62.5, 125, and 250 ppb GA vapour. The respective 2-year survival rates were 31/50, 27/50, 40/50, and 38/50 for males and 34/50, 37/50, 35/50, and 32/50 for females. Major findings were nasal histopathological effects, which were qualitatively similar to those for the rats. The incidence of respiratory epithelial squamous metaplasia was increased in the 250 ppb males and females and the 125 ppb females. Respiratory epithelial hyaline degeneration was increased significantly in all groups of GA-exposed females, but not in an exposure-related fashion. Nasal inflammation was increased in 250 ppb females. Turbinate necrosis was seen in 2/50 of the 125 ppb males and in all groups of exposed females. For rats and mice there was no other non-neoplastic histopathology. Under the conditions of this study, there was no evidence for an oncogenic potential with GA and no evidence for systemic toxicity.

Genetic toxicology

Numerous *in vitro* and *in vivo* genetic toxicology studies have been conducted, and summarized by Ballantyne and Jordan (2001). Mutagenic activity, generally weak, has been demonstrated in *Salmonella typhimurium* strains TA100, TA102, TA104, TA1535/PSK1002, BA9, and BA13 and in *Escherichia coli* strain WP2uvrA. Results with *in vitro* Chinese hamster ovary (CHO) cells depend on the gene locus investigated, with no activity being seen at the hypoxanthine–guanine–phosphoribosyl transferase (HGPRT) locus and weak activity at the thymidine kinase (TK) locus. *In vitro* increases in sister chromatid exchanges generally do not occur, and chromosomal aberration tests vary from no activity to weak activity. DNA damage and repair tests have shown variable results. *In vivo* studies generally have shown no evidence for genotoxic activity (micronucleus, chromosomal aberration, dominant lethal and *Drosophila* tests; Vergnes and Ballantyne, 2002). The absence of genotoxic effects *in vivo* may, in part, be related to the rapid metabolism and protein binding characteristics of GA, and the fact that ^{14}[C]GA cannot be detected in nuclei (Ranly, Amstutz, and Hern, 1990).

Developmental and reproductive toxicity

Several developmental toxicology studies have been conducted in various species. Mice given GA by gavage on gestational days (gd) 6–15 over a dosage range of

16–100 mg kg^{-1} day^{-1} showed maternal toxicity (mortality and decreased body weight) at 50 and 100 mg kg^{-1} day^{-1} with some indication of fetotoxicity (fetal body weight). However, fetotoxicity and teratogenic effects were not seen at maternally non-toxic doses (Marks, Worth, and Staples, 1980). With rats given GA by gavage at daily doses of 25, 50, or 100 mg/kg over gd 6–15, maternal mortality occurred at 50 and 100 mg/kg and body weight gain was reduced at 100 mg/kg. There were no effects on implantations, resorptions, or number of live fetuses. Fetal bodyweight was reduced at 100 mg/kg, but malformations were not seen (Ema, Itami, and Kawasaki, 1992). In another rat peroral developmental toxicity study, pregnant Wistar rats were given GA in drinking water at average daily dosages of 5.2, 25.7, and 68.0 mg/kg over gd 6–16. The only effect noted was a reduction in drinking water consumption at the mid and high dosages (Ballantyne and Jordan, 2001). In a rabbit study GA was given by gavage at daily dosages of 5, 15, or 45 mg/kg over gd 7–19. At the highest dosage there were signs of maternal and embryofetal toxicity, but no malformations. Neither maternal nor developmental toxicity were present at the mid and low dosages (Ballantyne and Jordan, 2001). With respect to reproductive toxicity, several subchronic repeated exposure toxicity studies have not shown any toxicity to the male or female reproductive tract. Also, a dominant lethal assay in mice by gavage showed no evidence for reduced fertility and no effect on embryofetal viability (Tamada *et al.*, 1978). In a definitive reproduction study, rats received GA in drinking water over two generations at 50, 250, or 500 ppm. This resulted only in a dosage-related decrease in parental water consumption and body weight, with no effects on reproductive performance (Neeper-Bradley and Ballantyne, 2000). Also, two epidemiological studies in hospital workers showed that exposure to GA was not associated with a risk of spontaneous abortion or other risks to reproductive performance (Hemminki *et al.*, 1982; Hemminki, Kyyonew, and Lindbohm, 1985).

Toxicokinetics and metabolism

An *in vivo* comparative intravenous and epicutaneous study was carried out using rats and rabbits (McKelvey *et al.*, 1992). By cutaneous application, a high proportion of [^{14}C]GA was recovered from the skin (45–61 per cent in the rat, 31–45 per cent in the rabbit). Autoradiography showed activity over the stratum corneum, hair shafts, and at foci of dermal necrosis. The proportion of GA absorbed percutaneously in the rat was calculated at 0.3–2.1 per cent, and in the rabbit at 7.5–24.9 per cent. Toxicokinetic profiles showed a bi-exponential form, suggesting a two-compartment model. The absorption rate constants were low in both species (range 0.2–2.0 h^{-1}). That the rat is a more appropriate model for the human, and shows low skin penetration, is strongly suggested by comparative *in vitro* studies. With isolated human skin it was found that GA did not penetrate isolated thick stratum corneum (sole of foot), whereas 2.8–4.4 per cent of GA penetrated isolated abdominal skin epidermis and 3.3–13.8 per cent penetrated isolated thin stratum

corneum from chest and abdominal skin (Reifenrath *et al.*, 1985). A detailed *in vitro* species comparison (rat, mouse, guinea pig, rabbit, and human) was undertaken with [1,5-^{14}C]GA (Frantz *et al.*, 1993). Highest recovery was with the skin of male mice (1.73 per cent with 0.75 per cent GA) and lowest with rat (0.05–0.06 per cent for females and males). Human female skin was low; 0.2 per cent recovery with 7.5 per cent GA and 0.16 per cent with 0.75 per cent GA. Glutaraldehyde is metabolized extensively to CO_2, probably through a series of oxidation, decarboxylayion, and hydrolysis reactions (Beauchamp *et al.*, 1992; Hjelle and Peterson, 1983; Karp, Korb, and Pashley, 1987; Myers *et al.*, 1986; Ranly, Amstutz, and Hern, 1990).

Human occupational medical features

The use patterns of GA imply a potential for human contact with the liquid on skin and eye, as well as exposure to vapour. Concentration-related skin and eye irritation, and allergic contact dermatitis, are well known over-exposure effects and are discussed earlier in the chapter. Sensory irritant and other symptoms have been documented extensively from exposure to the vapour (Binding and Witting, 1990; Jachuck *et al.*, 1989; Norback, 1988; Waters *et al.*, 2003). Typical are eye discomfort, excess lacrimation, blepharospasm, blepharitis, rhinorrhea, irritation and dryness of the throat, sneezing, coughing, slight breathing difficulties, headache, nausea, and fatigue. These effects are reversible within a few minutes to a few hours of the cessation of exposure. Epistaxis has been reported following vapour exposure (Wiggins, McCurdy, and Zeidenberg, 1989), and allergic contact dermatitis from vapour contact with skin (Fowler, 1989). The occurrence of asthmatic symptoms has been discussed previously. Additionally, inadequately decontaminated endoscopes and medical instruments may be a source of exposure for patients, and lead to inflammatory complications (reviewed by Ballantyne and Jordan, 2001). This has been noted especially for colitis and proctocolitis (Burtin *et al.*, 1993; McHanson *et al.*, 1998; Zissin, Gayer, and Maor-Kendler, 1999). Also, a potential has been noted for synovitis (Harner *et al.*, 1989), dysphagia (Isserow *et al.*, 1998; Leudtke *et al.*, 2003), and laryngotracheitis (Belani and Priedkains, 1977).

The threshold limit value (TLV) for GA is 0.05 ppm as a ceiling value, with irritation and sensitization notations (ACGIH, 2001). The UK Health and Safety Executive have established a maximum exposure limit (MEL) of 0.05 ppm (Evans *et al.*, 1997). Inasmuch as the odour threshold (0.04 ppm) is significantly lower than these values, it is likely that GA vapour will be detected by smell before reaching an over-exposure concentration.

Ecotoxicology

GA is hydrolysable with a DT_{50} of 508 days at pH 5.0, 102 days at pH 7.0, and 46 days at pH 9.0. Photolysis in water at pH 5.0 shows a DT_{50} of 196 days (Eriksson,

Johnson, and Tornlund, 1995). With a P_{ow} of 0.98, the potential for bioaccumulation is low. An aerobic metabolism study (water/sediment) indicated GA (10 mg/L) to be oxidized to glutaric acid. The $t_{1/2}$ was 10.6 h, indicating ready biodegradability. Toxicity to aquatic organisms has been determined as follows:

Scenedesmus subspicatus	72 h EC_{50} = 0.85 mg/L; NOEL = 0.31 mg/L
Daphnia magna	48 h EC_{50} = 16 mg/L
Leptomis maccochirus	96 h LC_{50} = 12 mg/L
Oncorrhynchus mykiss	96 h LC_{50} = 12 mg/L
Cyprinodon variegates	96 h LC_{50} = 31 mg/L (flow-through)
Crassostrea virginica	48 h LC_{50} = 0.55 mg/L
	96 h EC_{50} (shell growth) = 0.87 mg/L
	with NOEL = 0.16 mg/L
Carcinus maenus	LC_{50} = 465 mg/L
Palaemonetas bugio	LC_{50} = 41 mg/L

A reproductive study with *Daphnis magna* showed moderate toxicity with a 21-day NOEL of 2.1 mg/L. A predicted no-effect concentration (PNEC) of 6.2 μg/L for GA to aquatic ecosystems has been suggested by the Swedish National Chemicals Inspectorate (Eriksson, Johnson, and Tornlund, 1998).This is based on the lowest NOEL for the most sensitive strain (algae).

Extensive summaries of the toxicology, occupational medical, and industrial hygiene aspects of GA can be found in the following: Ballantyne and Jordan (2001); CIREP (1996); Evans *et al.* (1997); and Jordan (1995).

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Part IV

Residues

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10 Variability of Residues in Unprocessed Food Items and its Impact on Consumer Risk Assessment

Caroline A. Harris and **Alan R. C. Hill**

Variability of pesticide residues

As any analytical chemistry student should be able to tell you, the error associated with sampling can be vastly greater than that for analytical measurement. This is because, with very few exceptions, analytes are usually heterogeneously distributed in any material or population of materials. It is impossible to achieve a uniform deposition or distribution of a pesticidal active substance in something that is part of a diverse biological system and it is therefore not surprising that the concentration of pesticide residues occurring in individual units of fruit and vegetables tends to be very variable. In this chapter we define variability as the ratio of the residue level in the unit (a single fruit, vegetable, etc.) containing the highest concentration, divided by the arithmetic mean concentration in a composite sample from which the unit is drawn. The ratio is usually known as the variability factor. For most purposes in the post-registration control of pesticides, the sample is intended to be representative of the whole crop or batch of a commodity, etc. so the variability within the sample is also expected to be typical of the batch. Of course, 'typical' does not mean that the entire range of variation in concentrations will be present in the sample. Concentrations of pesticides usually vary within units of fruit and vegetables but the consequences of such variation for consumers is reasonably well understood, because the development of so-called 'processing factors' is nowadays usually a requirement for registration where measurable residues are known to occur in food at harvest. Ironically, the consequences of variability within tissues such as 'meat', of a single animal, are rather less well known.

It has always been recognized that residues in fruit and vegetable must vary but variability of pesticide deposition upon, or redistribution within, crops may have desirable or undesirable aspects, or both. For example, if poor penetration of a whole leaf canopy is achieved during spraying, there may be inadequate control of pests within the canopy. However, if the requirement is only to control a pest attacking the growing tips of the plant, complete penetration of the canopy may be wasteful of pesticide and lead to unnecessarily high residues in the harvested crop and the environment. In the latter case, variability in residue concentration is not unavoidable, it is intended and necessary.

However, it was not until the extent of residue variability in harvested crops was discovered in the United Kingdom in the early 1990s that the full implications began to become apparent for sampling and, particularly, for consumer risk assessment.

Maximum residue limits

Historically, levels of pesticide residues in foodstuffs have been compared with standards known as maximum residue limits (MRLs). MRLs are derived from field trials or animal feeding studies where crops (or animals) are treated according to good agricultural practice (GAP). GAP defines the maximum application rate and number of applications and minimum pre-harvest interval (PHI) or withholding period that may be required to give efficacious control of a specified pest in geographical areas of comparable soil and climatic conditions. GAP is always defined on the pesticide product label, so that the user can be in no doubt of the worst-case limits for application of the pesticide. Within these limits, governments, advisory agencies, product labels, and the whole principle of GAP encourage users to apply the minimum quantities of pesticides and the maximum harvest/withholding intervals. In most industrialized countries, representative worst-case GAP trials must be carried out prior to registration of a pesticide for use and the MRL represents the highest level that should occur following worst-case permitted GAP.

In the case of pesticides used as veterinary medicines, or which may occur as residues in treated animal feedingstuffs, the total dose given to individual large farm/food-producing animals is predictable within close limits. For this reason it is, fortunately, possible to utilize only a small number of animals in studies against GAP. For the same reason, MRLs for edible animal tissues or products generally are based on the highest residues found in an individual animal (with the predicted intake based on either median or highest dose/intake depending on the speed with which a steady state of residues is achieved within the animal). The MRLs may differ according to the part of the animal (e.g. fat, muscle, liver, kidney), dependent on the distribution and metabolism of residues in the animal. The range of farmed food animals is very small (with the possible exception of fish, shellfish, and crustaceans, for which there are only a very limited number of MRLs).

In the case of plant crops, whether treated in the field or post-harvest, the trials are necessarily carried out on a scale proportional to commercial production and the dose that arrives on/in the harvested/remaining parts is much less predictable. For this reason, composite samples are taken to determine the average levels of residues.

Minor crops

In contrast to animals, an enormous range of plant species is farmed for food and many of these may be described loosely as 'minor crops'. A full discussion of major and minor crops is beyond the scope of this chapter. However, minor crops are often produced in such small quantities that the cost of residue and other trials cannot be supported, or supported fully, by the value of the crop production. To avoid unnecessary and undesirable restriction of diets and consumer choice of food, whilst at the same time enabling good quality food to be produced, data generated for the purpose of fixing MRLs on major crops may be used to support MRLs for minor crops by extrapolation. For example, residues data generated using spinach or lettuce may be extrapolated to a wide range of leafy herb crops. Work has been funded by the European Commission and the OECD (Organization for Economic Co-operation and Development) to try to develop internationally accepted minimum requirements for establishing MRLs (Harris and Pim, 2000). Although some progress has been achieved, it has proven difficult to extend the guidance on extrapolation of residues data which would lead to a reduction in the numbers of residues trials required to set MRLs, particularly for minor crops.

Derivation of MRLs

MRLs are estimates, usually based on the highest residue concentration observed in single animals or in composite samples from trials on plant crops, but statistical calculations can also be used (SANCO, 1997). The values designated for MRLs follow a roughly geometric progression of usually 0.01, 0.02, 0.05, 0.1, 0.2, 0.3, 0.5, 1, 2, 5, 10, 25, or 50 mg/kg. In most cases where the residues are of no toxicological concern, the value encompassing the highest concentration found in the trials is designated as the MRL. This range has recently been reviewed and extended by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) to include values intermediate to this range (WHO/FAO, 2001) with the adoption of a standard 1, 2, 3, 5, 7 system for residue values up to 10 mg/kg, i.e. 0.01, 0.02, 0.03, 0.05, 0.07, 0.1, 0.2, 0.3, 0.5, 0.7, 1, 2, 3, 5, 7, and 10 mg/kg. For higher values, 15, 20, and 25 mg/kg have been found useful, and, up to 50 mg/kg, rounding to the next 10, i.e. 30, 40, 50, etc. is preferred. The option to use other values as necessary would be maintained by JMPR. Therefore, if crops or animals have been treated according to GAP, then residues detected in the food should

not, in principle, exceed the MRL. In practice, MRLs are very rarely exceeded following adherence to GAP, in part because the majority of pesticide applications do not approach the worst-case permissible. In very rare cases, changes in agricultural practice may lead to a worst-case scenario which was not envisaged when the MRL was set, with the consequences that the MRL may be exceeded more frequently. The few known cases have always been addressed as a priority by registration authorities and resolved.

Discovery of residue variability in UK crops

It was exceedances of an MRL that led to studies in the United Kingdom showing the extent of variability in residue concentrations between individual units of fruit and vegetables (Hill, 2000). During routine monitoring of residues in the UK food supply in the early 1990s, a consistent proportion of UK-grown carrots was found to contain residues above MRLs for certain organophosphorus pesticides, and in particular triazophos (Harris, 2000). These pesticides were used to control carrot fly, a very damaging pest of umbelliferous plants whose larvae can render carrot and parsnip roots unsaleable and effectively inedible.

Examination of registration data indicated that the Codex MRL for triazophos (upon which the UK MRL had been based) did not fully accommodate the residue levels that were likely to occur when adhering to the label recommendations in the United Kingdom. The minimum PHI had been based on treatment of crops early in the growing season, long before lifting for sale or storage. Storage is essential to extend the season of supply of these important vegetables beyond the summer and autumn months. Carrots can be, and are, maintained in cold stores in continental Europe but the practice is expensive (and thus the carrots are expensive) and the quality and appearance of the stored roots is not particularly good. The cool but not excessively cold climate in the United Kingdom lends itself to storage outside during winter. In former times, such storage was in clamps but these are very labour intensive and tend to generate damaged roots. In consequence, simple covering systems were developed to enable the roots to be stored in the field. The costs involved are low and root quality is maintained at a very high level – as long as carrot fly is well controlled. Application of organophosphorus pesticides in the late summer and early autumn not only provided good control of carrot fly but the timing of the applications easily complied with the minimum PHIs. Triazophos was one of the most popular pesticides for this purpose but the MRL of 0.1 mg/kg was based on applications made early in the growing season, when the carrots were small and growing rapidly in relatively high temperatures. The treatments for overwintering were made when crop growth was slowing and temperatures were low. The residues declined little during the winter and, in consequence, tended to exceed the MRL when roots were lifted in the spring. The situation may have been exacerbated by the dry weather experienced in the autumns of the early 1990s,

particularly as these conditions favoured the carrot fly, markedly increasing the need for control measures.

A new MRL of 1 mg/kg for triazophos was therefore adopted in the United Kingdom, based on GAP and registration data. The following season, residues of triazophos did not exceed the new MRL but residues of some other pesticides exceeded their respective MRLs even though, notionally, they took account of UK GAP (Harris, 2000).

To investigate the problem, carrots of known treatment history were collected directly from commercial and experimental farms. Samples were taken in such a way as to allow the examination of as many geographical areas, different crop varieties, cultural regimes, treatment regimes, and soil types as possible. All carefully recorded crop treatment histories indicated that GAP had been observed. There was no correlation between residue levels and any cultural, geographic, or other factor which might have been linked to them. A striking feature of the few cases where samples had been taken from adjoining fields, subjected to identical cultural regimes, was the large differences in residue levels observed.

Whilst this work was progressing, the effects of domestic processing (such as topping, peeling, and cooking) on residue levels in carrots were also being studied. It was quickly established that the residues of (mostly poorly systemic) organophosphorus pesticides were most concentrated in the root crowns and/or peel but, given the methods of application in the field, this was not surprising. In contrast with this simple interpretation, the data from cooking experiments were highly variable. Where levels were expected to decline, in some cases they remained constant but in others they increased. The analytical methods were of good precision, and therefore not the source of this variation, and the variations found in cooking were too great to derive from variations introduced by inconsistency in topping and peeling. An attempt to determine whether residue levels were related to the size of individual carrots showed that there was no correlation but showed, coincidentally, that the differences in residue levels in roots from the same sample were extremely large in many cases. This observation offered a simple explanation for all the difficulties previously experienced in trying to reconcile disparate results from notionally similar samples. That is, the problems were due to residue variability, which manifested itself through sampling error.

Further studies on variability

Studies of residue variability conducted during the mid- to late 1990s produced a large database on carrots (PSD, 1994, 1995, 1997), apples, pears, oranges, tomatoes, bananas, peaches, and nectarines in 1996 (Advisory Committee on Pesticides, 1997), kiwi fruit, plums, and celery in 1997 (PSD, 1998), and potatoes in 1999 (PSD, 1999a). Data were generated on a wide range of pesticides, with special emphasis on organophosphorus and carbamate compounds, because of their acute

toxicity. Other data have been published recently including phosphamidon or chlorpyrifos-methyl in apples, chlorpyrifos, pirimiphos-methyl or vinclozolin in kiwi fruit, an unnamed pesticide applied as granules to potatoes (Ambrus, 2000), thiabendazole in pears, methamidophos, procymidone, and endosulfan in sweet peppers, diphenylamine and phosalone in apples, ethion and chlorothalonil in bunches of parsley, methamidophos in cucumbers, prothiofos in oranges and monocrotophos in individual bunches of grapes (Anderson, 2000), unspecified pesticides in oranges, peaches, carrots (Earl, Kaethner, and Uihlein, 2000), and aldicarb in oranges (Lentza-Rizos and Tsioumplekou, 2001). Monitoring programmes have also incorporated individual unit analysis into their schedule, e.g. the European Union (SANCO, 2000) and the United States (USDA, 2001).

Implications of variability and derivation of the variability factor

Why is variability such an important phenomenon? If we ignore the issue of determination of compliance with MRLs, the main reason is that some individual units must contain higher residues than the average measured in a composite sample. This is not critical in cases where many individual small units are consumed (e.g. peas), or where many units are blended in processing (e.g. wheat for bread). In such cases, exposure to residues for most consumers of the food from a particular batch is likely to be fairly similar to that indicated by the average concentration. In contrast, if only a single large item is consumed (e.g. an apple), the average value provides little indication of the maximum possible intake by a single consumer. Thus there may be an under-estimation of consumer exposure and risk if the residue is of an acutely toxic pesticide.

Until the information on residue variability emerged, generally only long-term, or chronic, exposure to pesticide residues had been taken into account during the registration or review process for pesticides. Residue variability is not an important issue in assessing the risks posed by pesticides of chronic toxicity because the variability is averaged out by repeated consumption. That is, the probability of any person consuming only those units that contain the highest residue level throughout life is so low as to make it impossible. In fact, an impossibly low probability is reached within a small number of meals.

The variability data generated in the United Kingdom in the 1990s provided sufficient information on residues in individual units to calculate 'acute' intakes for consumers, i.e. relating to a single day's exposure. Triazophos and carbaryl residues in apples were shown to give rise to intakes above the acute reference dose (acute RfD) at the 97.5th percentile of probability (Hamey and Harris, 1999). However, although a large number of data on unit to unit variability had become available, it was unclear how variability should be built into calculations to estimate

the acceptability of residues, in the many cases where sufficient data did not exist. A joint FAO/WHO consultation recognized that acute exposure of consumers was important and should be assessed (WHO, 1996). A second FAO/WHO consultation meeting recommended values which should be used as variability factors in the absence of definitive data (WHO, 1997). The recommendations were based primarily on the numbers of units taken as a composite sample, giving a variability factor, v, of 1 for commodities comprised of small sized units, such as pulses, grain or fruit and vegetables <25 g, 5 for large sized commodities (unit weight >250 g in melons), or 10 for medium sized commodities (unit weights 25–250 g, i.e. apples). The basis of the v values 5 and 10 was that the Codex recommendations for sampling these products are that a sample must be comprised of a minimum of 5 or 10 units. In these cases, the most extreme variability theoretically possible in these cases derives from the entire measured residue being present in just one of the units. In practice, this is highly improbable, except where treated and untreated units have been mixed and even here the probability is low. Nonetheless, the basis for the $v = 1$ value may appear contrary, when the Codex sampling recommendation is to take a minimum of 1 kg and the minimum number of units is therefore 40. However, in this case, the value of 1 represents an 'infinitely' small probability that a consumer could obtain in one meal, from, say, 1kg of frozen peas, a portion of perhaps 100 peas containing only those with the highest residues. There is a high probability that residue levels in the other peas in the portion that actually contains the pea with the highest residue will be such as to make the concentration in the portion rather similar to that of the average concentration in the 1 kg sample. In 1998, an international conference re-examined the variability distributions and confirmed the 10 factor as a worst-case scenario (Harris *et al.*, 2000). On the basis of the variability data, the factor for medium-sized commodities became 7 except for soil-applied, granular formulations where insufficient data were available for assessment and therefore this value was maintained at 10. Further data were made available to the JMPR in 2002 which allowed it to recommend an additional variability factor of 3 for head-lettuce and head-cabbage (FAO, 2002).

Calculation of acute dietary exposure

The proposed variability factors were intended to be used in calculations known as international or national estimates of short-term intake (IESTI or NESTI).

One of the aims of WHO/FAO during their series of consultation meetings had been to refine calculations, to try to avoid some of the more extreme sources of over-estimation that are associated with calculating consumer exposure to pesticides, without reducing margins of safety for consumers. In this particular case the IESTIs/NESTIs should therefore take into account that not all units in the portion consumed could possibly contain residues at the highest level. The assumption was made that, where more than one unit of a fruit or vegetable was consumed over a

Table 10.1 Definitions of abbreviations used for estimating acute or short-term consumer exposures, as presented on pp. 420–422 (after JMPR, WHO/FAO, 2001)

Abbreviation	Definition
LP	Large portion size, kg/day (97.5th percentile of consumption of those who consume the particular food).
HR	Highest residue level (mg/kg) in the edible portion of composite samples analysed for supervised trials, which support the MRL and STMR.
Bw	Average consumer body weight (kg) provided by the country and consumer population from which the large portion (LP) size was used.
U	Unit weight in trials which produced the highest residue level (HR) supporting the MRL. Unit weights consist of two components, the weight of a typical commodity unit in trade and the per cent edible portion.
v	Variability factor. The 97.5th percentile of residue levels occurring in single units of a lot divided by the arithmetic mean residue for the lot for samples taken from controlled trials.
STMR	Supervised trials median residue (mg/kg), notionally representing the typical residue level in edible portion resulting from worst-case GAP.
STMR-P	The STMR for a processed commodity, calculated from STMR for the raw commodity and the processing factor.

day, if the first unit consumed contained residues at a level equivalent to the highest residue level (HR) found in any of the samples taken from supervised trials, multiplied by the variability factor, *v*, then any subsequent units or parts of units would contain residues at the median residue level observed in samples from the supervised trials (STMR). This clearly remains an over-estimate because, if the first unit consumed contains the entire residue in the sample (as defined by *v*) which represents the highest level produced by worst-case GAP, then the second or subsequent units cannot possibly contain residues at the STMR. Nonetheless, this estimate gave rise to a series of calculations to take account of the different scenarios that could, theoretically, exist.

Calculations follow that were derived by the JMPR (WHO/FAO, 2001) for the estimation of acute or short-term consumer exposure (IESTIs/NESTIs). Abbreviations are as in Table 10.1.

Case 1

In the first case, residue data obtained from the usual composite (multiple-unit) samples reflect the residue level in a meal-sized portion of the food (commodity unit

weight is below 25 g). The residue intake is simply the large portion size (LP, in kg) divided by the average consumer body weight (bw, in kg) and multiplied by the highest residue level (HR, in mg/kg) in the edible portion found in the supervised trials at maximum registered use, i.e. according to GAP and supporting the MRL:

$$\text{IESTI/NESTI} = \frac{\text{LP} \times \text{HR}}{\text{Bw}}$$

Case 2

In Case 2, the residue data from composite samples do not reflect the residue level in a meal-sized portion of the food (raw commodity unit weight exceeds 25 g). Different calculations are utilized, depending on whether the unit weight (that is, the edible portion of the unit) is less than or greater than the large portion size.

Case 2a

In Case 2a, the unit weight of the raw commodity (edible portion) is less than the large portion weight. The assumption is that the first unit consumed contains the highest residue level, i.e. the residue level in the unit equals the residue level in the composite sample multiplied by the variability factor. The remainder of the large portion then contains residues at the STMR.

The 2000 JMPR (WHO/FAO, 2001) noted that this approach was based on the assumption that the units consumed were from a population composed of different lots. However, it is likely that the units supplied together have originated from a single lot and form the composite sample, and in that case the second part of the equation, which accounts for consumption of the second and subsequent units, making up the remainder of the large portion, should have the STMR replaced by the HR. The Case 2a formula then becomes:

$$\text{IESTI/NESTI} = \frac{\text{U} \times \text{HR} \times v + (\text{LP} - \text{U}) \times \text{HR}}{\text{Bw}}$$

Case 2b

In Case 2b, the unit weight of raw commodity (edible portion) exceeds the large portion weight, so the residue level in the large portion equals the residue level in the unit containing HR multiplied by the variability factor.

$$\text{IESTI/NESTI} = \frac{\text{LP} \times \text{HR} \times v}{\text{Bw}}$$

Case 3

Case 3 is used for processed commodities, where bulking or blending means that the STMR-P (the supervised median trial residue adjusted for a changes in residue concentration as a result of processing) represents the likely highest residue. Such examples are fruit juices, vegetable oils, sugar, and flour, where processing is on a large scale and where raw commodities from a number of farms contribute to the final product. Generally, processing leads to a reduction in residue levels although there are some exceptions, such as the increased concentration of lipophilic pesticides in expressed/extracted oils and fats or of grain protectants in bran from treated cereal grains.

The United Kingdom and the European Union have adopted a methodology similar to that used by the JMPR for calculating acute point estimates of intake (PSD, 1999b). Parameters such as portion sizes, body weights, etc. are taken directly from UK surveys (Gregory *et al.*, 1990; Gregory *et al.*, 1995). The main differences exist in terms of numbering the calculations. The UK Case 3 calculation is designed for those situations where data on individual units are available, which is a different situation from the JMPR Case 3 calculation for processed commodities.

Refinement of the variability factor

Following development of this necessarily crude approach by FAO/WHO, efforts have been made to refine estimates of v for various commodities. At an international conference on pesticide residue variability and acute dietary risk assessment held in the United Kingdom in 1998, a working group, studying the causes of variability and related sampling issues, re-examined the UK data sets to try to refine v (Harris *et al.*, 2000). They recommended that, where sufficient data on residues in individual units exist, v should be calculated as the 97.5th percentile of residue levels found in individual units (or in a single serving portion for large or small unit crops), as opposed to the HR previously divided by the sample mean used. The 97.5th percentile (the arithmetic mean plus 2 standard deviations) was chosen as the most extreme value that can be estimated with any reasonable degree of certainty from a limited set of data. In principle, the 100th percentile can be determined only by measuring the residues in every single unit of a crop or batch – an impossibly costly and wasteful exercise that would leave no food for consumers.

However, the working group considered that insufficient data were available to refine the FAO/WHO values for v and noted that several units of certain crops, such as strawberries or grapes, would usually be consumed in a single serving and may require the derivation of a suitable v, which would be different from those adopted for crops associated with large-sized units. Also, the first residue term in the Case 2a equation, MRL-p (the maximum residue level adjusted for a changes in residue

concentration as a result of processing), was considered to contribute to the over-estimate associated with point estimates due to the geometric progression used in setting MRLs. This term should therefore be replaced by HR-P (highest residue measured in composite samples from supervised field trials with any changes in levels of residues due to processing being taken into account).

A project undertaken by the International Union of Pure and Applied Chemistry (IUPAC) is currently reassessing all the available sets of variability data, using only those data from samples in which a high proportion of units contained detectable residues (>90 per cent) and re-examining these to consider whether v can be further refined for those crops (IUPAC, 2002). The findings are due to be published in 2003.

Consumption data in assessing acute dietary exposure

In calculating acute (short-term or daily, in effect) exposure to pesticide residues, the use of lifetime average food consumption data is clearly not appropriate. Such 'chronic' data even out the peaks, troughs, and changes in consumption patterns that inevitably occur throughout life. Long-term consumption data take account of days on which none of a particular commodity is consumed, which is not appropriate for worst-case estimates based on consumption in a single meal or day. Therefore food consumption data for a particular foodstuff required for calculating chronic exposures will necessarily be lower than those used to calculate food consumption on a single day. Consumption data for a single day are termed 'portion size' and are used for calculating acute exposure to pesticides and other toxic chemicals. In the United Kingdom, two data bases are used to determine estimates of portion size, for adults (Gregory *et al.*, 1990) and 'toddlers' (children aged 1½–4½ years) (Gregory *et al.*, 1995). These databases are generally considered to include the extremes of consumption, taking account of the high consumption to body weight ratio for toddlers over a generally limited range of foods, versus the wider range of foods likely to be available to and consumed by adults. Thus the use of additional databases is unlikely to produce additional useful information. Unlike toddlers, who may consume fruit or vegetables as intact units, infants are most likely to consume food which has been homogenized (e.g. purées). The consequential elimination of residue variability means that the variability factor, v, is usually taken to be 1 for the infant consumer group.

Other agencies, such as the USEPA, utilize food consumption databases which are divided into a wider range of consumer groups, taking account of factors such as age, sex, and ethnicity (National Centre for Health Statistics, 2003). Other data sets are now being assembled, based on large portion size information for use at an international level (WHO, 2002). Typically the 97.5th percentile value has been

chosen to represent 'high level' consumers but not the extreme consumer of food. The basis for using this percentile is not well established, although in the long-term consumption of frequently consumed foods, it generally represents the point at which a 'high plateau' value is obtained. By default, perhaps, this percentile has also been used in assessments of short-term exposure. Debate continues on the appropriate percentile with the United States using 99.9th percentile as a decision-making point in consumer risk assessment (Chaisson, Sielken, and Waylett, 1999; Julien *et al.*, 2001).

Toxicology in the derivation of an acute reference dose

For assessment of the potential impact of acute exposure to pesticide residues, the usual toxicological end-point that determines the acceptable daily intake (ADI) was not considered appropriate. The ADI was devised to deal with consumer exposure to residues over a lifetime rather than in a single meal or a day. Instead, a value termed the acute reference dose (acute RfD) was derived from available toxicological studies as the appropriate value for assessing the risks associated with acute exposure. Unfortunately, most of the current 'standard' toxicology studies are not directly aimed at the derivation of an acute RfD and, mainly due to the dose levels used in the studies, are likely to give excessively cautious values in most cases (Dewhurst, 2000). Whilst many single dose, acute exposure studies are performed, such as the LD_{50} test, such tests are not specifically designed to identify sub-lethal effects from oral intake. The most commonly used studies to derive acute RfDs are teratology studies (in which a toxic influence introduced at a single point in time leads to a permanent effect in the offspring) or those that include measurements of metabolic or physiological changes such as cholinesterase inhibition (in which the toxicological effect may be transient). It has been noted that range-finding studies may represent a currently unused source of relevant data as they contain information on specific effects which may be relevant to the toxicological end-point of interest. An OECD guideline for studies intended to produce data relevant to the derivation of an acute RfD is currently under consideration. In a manner analogous to the derivation of an ADI, the appropriate 'no observable adverse effect level' (NOAEL) for acute effects is divided by an appropriate safety factor, typically 10–500 depending on the severity of the effect observed (end-point), to obtain an acute RfD. The assessment of consumer risk from acute exposure to pesticide residues involves comparison of the calculated intake of residues, in mg/kg bw/day, against the acute RfD expressed in the same units. If the calculated intake does not exceed the acute RfD (which, as indicated above, incorporates a 10–500-fold safety margin) then, for the purposes of regulating pesticide use, the risk is considered acceptable. Acute RfDs are not always necessary as some compounds may not

display acute or short-term effects. If the toxicological data do not justify the derivation of an acute RfD, then it is not usually considered necessary to carry out an acute or short-term consumer exposure calculation. Where an acute exposure is likely to be toxicologically relevant but insufficient data exist to derive an acute RfD, then the ADI (which will not be higher than the acute RfD) is used as a surrogate (WHO/FAO, 2002). The use of the ADI in this case is precautionary and can be expected to lead to an over-estimate of acute risks, because any organism can tolerate almost any adverse physical or chemical influence at a higher dose for a short time than if it is exposed to the same influence throughout its life. The only exceptions to this rule relate to teratogenic effects, for which continued exposure through adult life may be of little consequence to the individual, in comparison with short-term exposure during gestation.

Calculating consumer exposure

Whilst deterministic or point estimates, i.e. using a calculation based on a single value to represent residue, consumption, and body weight, remain the simplest and quickest method of estimating consumer acute exposure to pesticide residues, they lead to gross over-estimates, because worst-case data must be applied throughout. To allow more rational estimates of risk from acutely toxic compounds, probabilistic models are now being developed that allow the use of ranges (or, preferably, the complete frequency distributions) of data such as food consumption or residues and take into account both the changes in residue levels due to food processing and the real use patterns of pesticides. In fact, any appropriate variable that can be modelled as a discrete or continuous distribution can be included in such models. Instead of producing a simplistic single and unrealistically extreme value, as in deterministic models, residue intake can be presented as a range of values where any percentile of consumers can be chosen as a cut-off point for regulatory decision-making. Outputs, expressed as the proportion of consumers likely exceed a certain limit, are also possible. Debate continues about the appropriate percentile to use for decision-making, although the United States has chosen the 99.9th percentile (Chaisson *et al*., 1999). However, it must be emphasized that the errors involved in estimates at the extremes are inherently much greater than those at less extreme points. For this reason, the volume of data required to derive a sound value for a 99th percentile is much greater than that required to provide the same degree of assurance for, say, the 97.5th or the 95th percentiles.

A practical difficulty in probabilistic modelling is that whilst commercially available programs exist such as '@ Risk' (Palisade) or 'Crystal Ball' (Decisioneering), they take a reasonable amount of computing power and, especially, large and robust data sets to use in the model. Consequently, they demand considerably more resources than deterministic estimates. Whilst the FAO and the WHO have recognized that probabilistic modelling is likely to become more accessible in the future, there are currently

very few agencies in the world that are able to carry out such assessments (Harris, 2000). Commercial products such as DEEM^{TM} (Novigen Science Inc., 1999) are available or are under development (Monte Carlo under the EU's Fifth Framework programme [IEFS, 2003]) but it is still likely to be some time before sufficient consumption data and associated recipes are available to justify the routine use of such techniques in consumer risk assessments. Whilst, on a scientific basis, many agencies can accept probabilistic modelling techniques, politically and ethically it is difficult to accept the use of any pesticide which may give rise to any exposure that may exceed the acute RfD even for a very small percentage of consumers. For example, the 99.99th percentile – which is virtually impossible to determine accurately because of the costs of generating the data – excludes 1 person in 10 000, which equates to a large number of people in anything but the smallest countries of the world. The probabilistic modelling technique is also sometimes viewed as a way of reducing the apparent seriousness of an unacceptable risk (Lefferts, 2000). However, technically, if enough simulations have been carried out using the same datasets, then the extreme values seen in deterministic estimates should also occur as part of the output in the probabilistic modelling.

The impact on availability of pesticides

Since the introduction of acute risk assessments in the United Kingodm and by the FAO/WHO JMPR, uses of several pesticides have been shown to give calculated residue intakes by consumers that exceed the acute RfD. In the Codex Committee on Pesticide Residues, MRLs are now not being accepted by member governments where there are acute RfD exceedances based on a deterministic or point estimate basis. Inevitably, pesticides giving rise to unacceptable acute exposure of consumers will be withdrawn either by manufacturers who cannot justify the expense of producing data which would allow valid refinements to be made to the exposure calculations, or acute RfD, or by regulatory agencies. Ultimately, this will reduce the range of pesticides available to farmers and is likely to be reflected in the price paid by consumers for the food products and probably in the range or quality of food available. Whilst probabilistic modelling is currently an expensive and labour-intensive methodology, it may prevent the loss of some highly effective and important pesticides from the market in the future.

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Part V

Human Aspects

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11 Occupational Aspects of Pesticide Toxicity in Humans

Angelo Moretto

Occurrence of occupational pesticide poisoning

Statistical information on occupational pesticide poisoning is difficult to obtain because of several factors. These include misdiagnosis, under-reporting, and the difficulty of distinguishing occupational from other accidental poisonings and purposeful self-poisoning (Wesseling *et al.*, 1997a). The California Pesticide Illness Surveillance Programme, which enforces mandatory physician reporting of all occupational pesticide poisoning incidents, probably under-estimates the true rate of illness related to pesticide exposure. Based on the California data, it has been estimated that the physician-diagnosed pesticide occupational illnesses in the United States would be 20 000–40 000 per year, about 50 per cent involving agricultural workers (Blondell, 1997).

Estimates of pesticide poisoning world-wide have been produced by WHO/UNEP (1990): severe poisonings per year are estimated to be 3 000 000 (about 25 per cent occupational), with 220 000 deaths (about 6 per cent occupational), the majority occurring in developing tropical countries. Partially available data indicate that the percentage of occupational pesticide poisoning varies between countries. When compared with data from poison control centres, occupational pesticide poisonings (mild to severe, including 3 deaths in the years 1985–92) are 5–8 per cent in the United States (Blondell, 1997; Litovitz, Clark, and Soloway, 1994), 38 per cent in Costa Rica (Leveridge, 1998), about 25 per cent in the United Kingdom (Thompson, Casey and Vale, 1995), 11 per cent in South Africa (London *et al.*, 1994) of total pesticide poisonings.

Data on poisoning rates among pesticide exposed workers are also available for some individual countries. For instance, Jeyaratnam (1990) estimated that in four Asian countries, 3 per cent (about 25 million) of the pesticide exposed workers, might

have pesticide-related illness every year. In Sri Lanka, 7–22 per cent of farmers are reported to have suffered from a pesticide-related acute illness in the previous years (van der Hoek *et al.*, 1998). In the United States, among a cohort of more than 50 000 pesticide applicators, 16 per cent reported having a high pesticide exposure event (which did not always result in toxic effects) (Keim and Alavanja, 2001); within the same cohort, on the basis of a nested case–control analysis, it was estimated that about 0.6 per cent of the applicators had symptoms (Alavanja *et al.*, 2001). In Mexico, among 200 seasonal workers, 20 per cent experienced acute pesticide poisoning (De Jesus Chain-Castro, Barron-Aragon, and Haro-Garcia, 1998); in Costa Rica, an overall rate of pesticide poisoning of 5.3 per 100 workers per year was calculated based on data from the National Insurance Institute (Vergara and Fuortes, 1998); somewhat lower rates were reported by Wesseling *et al.* (2001).

Acceptable occupational exposure levels (AOELs) and estimate of levels of pesticide exposure

The Acceptable Occupational Exposure Level (AOEL) is defined by the Directive of the European Union 91/414 concerning the placing of plant protection products on the market as 'the maximum amount of active substance to which the operator may be exposed without any adverse health effect' and is expressed as mg/kg bw/day of absorbed (via any route of exposure) dose available for systemic distribution (CEC, 1991). AOELs for agricultural pesticides are derived from the toxicological database of the active substance involved. These levels are considered to be safe for workers exposed to the formulated product used as recommended. Therefore, worker exposure, either measured or estimated, must be compared with the established AOEL. In the European Union, an estimated exposure above the AOEL prevents the registration of the active ingredient or of some of its formulations and/or uses. It should be noted that AOELs are intended for pre-registration risk assessment purposes and not as a tool to control worker exposure. In this, AOELs differ from Occupational Exposure Limits (OELs) used in an industrial setting which are typically set as 8-h time-weighted averages, based on a working life-time of 40 h/week, or 15-min short-term exposure levels, and are used to monitor occupational exposure.

There is no general agreement so far on how to establish the AOEL because of the difficulty of defining the worker exposure conditions and, consequently, selecting the appropriate toxicological end-point and time-point of both experimental and human data (FAIR, 2000). This is in addition to the well-known problems related to the extrapolation of experimental animal data to humans. Working conditions of pesticide operators are different from those of industrial workers because they are intermittent, depend on climate and type of culture, on the equipment used, and entail a more significant dermal exposure rather than inhalation. It has been estimated that dermal exposure accounts for about 90 per cent of total exposure (Ross, Dong, and Krieger,

2000) and, therefore, it has to be taken into account when assessing overall exposure. For instance, the Americal Conference of Governmental Industrial Hygienists (ACGIH) establishes inhalation limits for many pesticides with skin notation (ACGIH, 2001). The rate of skin absorption is highly variable depending on the active ingredient, the solvent, the concentration, the skin area, and the length of exposure. In addition, the actual determination of worker exposure (absorbed dose) is difficult because of the lack of proper biomarkers for biological monitoring (see pertinent paragraphs) and because field studies are difficult and costly to perform. As a consequence, the use of predictive exposure models has become quite common and well established both in Europe and in the United States and a number of different models have been developed over the years (Hackathorn and Eberhart, 1985; Lundhen *et al.*, 1992; PHED, 1992; PSD, 1986; van Golsteins Brouwers, Marquat, and Hemmen, 1996; van Hemmen, 1993, 2001; van Hemmen, van Golsteins Brouwers, and Brouwers, 1995). In Europe, the introduction of Directive 91/414 required harmonization of procedures, including estimates of worker exposure. Consequently a group of experts from industry, government, and academia was given the task of developing a predictive occupational exposure model that would be applicable within EU countries (hence the acronym EUROPOEM) (van Hemmen, 2001). A database of field studies, either published or proprietary unpublished, was evaluated: these studies included separate exposure data during mixing, loading, or application, and exposure data for those activities performed by the same worker. In addition, studies were classified according to the formulation used (e.g. powders, granules, liquids), and according to the modality of application (e.g. upward, downward, tractor-driven, hand-held equipment). The rationale behind this exercise was the extrapolation across different active substances if use scenarios were similar (type of formulation and application modality), except for volatile compounds (defined as those having a vapour pressure above 10 mPa) for which corrections for inhalation exposure may be required. From each database for comparable exposure scenarios, surrogate potential exposure values were obtained, based on statistical consideration; for instance, when the database was considered adequate, the 75th percentile was used (van Hemmen, 2001). Otherwise the estimated values depended on the number of available data and expert judgement always came into play. In the early 1980s, in North America, a joint effort of the US Environmental Protection Agency (USEPA), Health (then Health and Welfare) Canada, and the America Crop Protection Association (then National Agricultural Chemical Association) began the development of a worker exposure database to be used for exposure assessment for regulatory decisions. In 1992, the first version of the Pesticide Handlers Exposure Database (PHED, 1992) was released. It provided dermal and inhalation exposure data for mixing/loading and application of pesticides. PHED allows the modelling and predicting of potential pesticide exposures based on different scenarios which take into account a number of variables such as application rate, formulation characteristics, methods and equipment for mixing/loading and application, and protective equipment. PHED has a number of limitations caused by the wide differences in the design, detection limits, and quality of the studies used for the database (Whitmyre, Ross, and Lunchick, 2001).

These caused a high variability of the data and also a high variability of non-detects which pose statistical problems in processing the data. In general, PHED uses the central tendency of the data. In addition, in the absence of actual measurements, PHED does not estimate the effects of clothing or other protective measures on dermal exposure. Clearly, PHED and EUROPOEM need enlarged databases which include data on exposure scenarios such as re-entry and bystanders.

A tiered approach is proposed to assess acceptability of worker exposure to a given active ingredient: in the first tier the most conservative estimate is obtained from worst-case assumptions for all relevant variables (application rates, volumes, concentration, and non-use of personal protective equipment). This estimate is compared with the appropriate AOEL: if it exceeds the AOEL, then in the second tier the use of protective equipment is taken into account, and corrections for dermal or inhalation absorption are made if applicable. If the estimate is still above the AOEL, then a field study, possibly with both ambient and biological monitoring, is needed as a third tier (OECD, 1997).

Agricultural re-entry workers encounter a hazard in the workplace that is markedly different from that of pesticide handlers and applicators. The presence of foliar or soil pesticide residues following application is the primary hazard with potential for significant dermal exposures during hand activities (Kissel and Fenske, 2000); other sources of exposure are the compound still present in air (vapours, aerosols, evaporation from crop or soil) or brought into the air by workers' activities. For these workers, acceptable exposure is frequently accomplished by the establishment of prolonged re-entry intervals (i.e. the time between application of the pesticide and entry of the workers into treated fields) to allow for a significant decay of residue concentration. No formally accepted model or database is available for estimation of exposure of these workers. The USEPA has guidelines for assessing post-application exposures (EPA, 1984) which take into account dislodgeable foliar residues, foliage-to-human transfer coefficients, and duration of activity. If applicable, dermal absorption factors are then applied to estimate the dose. However, the number of variables to be taken into account is so high that general rules are difficult to establish. In fact, the extent of exposure depends on the formulation and amount of pesticide applied, the time elapsed from application, and persistence of the active ingredient which are influenced by climatic conditions (Davies *et al.*, 1981; Nigg, Stamper, and Queen, 1984). Mathematical models to calculate re-entry times on the basis of estimated exposure, on the decay of the residues, and on the toxicological profile of the compound have been proposed but they are difficult to apply and have not been validated (Kissel and Fenske, 2000; Popendorf and Leffingwell, 1982; Zweig *et al.*, 1985).

A particular exposure to organophosphates (OPs), organochlorines (mostly in the past) and, more recently, to synthetic pyrethroids occurs in sheep-dippers. A model to predict the extent of exposure has been developed for workers exposed to OPs (Pilkington *et al.*, 1999) based on determination of urinary metabolites after exposure. It was found that most of the variability could be accounted for by the number of times the concentrate formulation was handled and a time-weighted splash score which accounted for contact with dip wash, effectiveness of protective

clothing, washing, smoking and eating habits, and any other significant incident. This model was used to assess the exposure of sheep farmers and dippers in an epidemiological study conducted in the United Kingdom (Pilkington *et al.*, 1999).

The main problem remaining to be solved is estimation of the dermal absorption rate by exposed workers, i.e. estimation of the percentage of absorption of the compound that is deposited on the skin. It has been claimed that the commonly used approach using rat *in vivo* and *in vitro* data introduces a high level of conservatism in exposure assessment (Ross, Dong, and Krieger, 2000). Steps in which conservatism might occur are: (a) The use of rat rather than human dermal absorption data; occasionally rat skin permeability resembles that of human skin, but discrepancies showing rat skin being from 2- to 10-fold more permeable than human skin are common. (b) The assumption that the compound that is found bound to the skin at the end of the experimental study is available for absorption; this is not always true and may lead up to a 3-fold over-estimation (Thongsinthusak *et al.*, 1999). (c) The use of very low concentrations in volunteer studies, lower than those encountered in field studies; there are indications that as the amount on the skin increases, the efficiency of absorption decreases up to 5-fold (Wester and Maibach, 1976). (d) The assumption that percentage of absorption and not its rate is relevant; it is known that many effects (e.g. inhibition of acetylcholinesterase by OPs) depends more on peak plasma concentration ($C_{\max}$) rather than on total amount absorbed (AUC); skin absorption usually is slower than oral and therefore lower $C_{\max}$ levels are expected from dermal exposure even if the total absorbed dose is the same (Auton, Ramsey, and Woollen, 1993; Carmichael *et al.*, 1989). These possibilities may combine leading to over-estimates which may be of one or more orders of magnitude. This will have significant consequences in risk management and communication, may cause unnecessary anxiety and confusion, and require excessive mitigation measures (Ross, Dong, and Krieger, 2000).

Although the dermal route is the primary occupational exposure route, there are some exceptions which include pesticides with high vapour pressures (e.g. fumigants) and when certain specialized equipment that generates aerosols of respirable diameter ($<20\,\mu$m) is used: here inhalation becomes relevant. However, in most cases respirable particulates generated during application of pesticides are <10 per cent of the total (Ross *et al.*, 2001). Therefore, particle size must be taken into account when evaluating inhalation toxicity studies. In many experimental studies in animals, particles $<10\,\mu$m in diameter are generated and this leads to an over-estimation of inhalation and absorption by the exposed worker.

Generalities on biological monitoring of pesticide exposure

Biological monitoring is the measurement of a chemical substance or its metabolite(s) in body tissues, secretions, excreta, or expired air, or determination of biochemical

interaction between the substance or its metabolite(s) with body molecules (enzymes, hormones, proteins). Biological markers usually are distinguished in three categories: biomarkers of exposure, effect, and susceptibility (IPCS, 1993).

A biomarker of exposure can be a chemical or its metabolite(s) or the product of an interaction between the active substance and body molecule(s) or cell(s) bearing no apparent (patho)physiological consequence. A biomarker of effect is a biochemical or physiological alteration that is associated with a potential or actual adverse health effect. A biomarker of susceptibility is defined as an indicator of a reduced ability of an organism to deal with the exposure to a xenobiotic. This reduced ability might either be genetically or environmentally determined. Although biological monitoring may provide useful information to complement ambient monitoring, it is not frequently used on workers exposed to pesticides. The main reasons are two-fold. On one hand, there are practical limitations due to the variable characteristics of exposure; often unpredictable and different compounds may be used on consecutive days or even during the same working day. On the other hand, there is only a limited number of validated biological indicators.

The most commonly used body fluids, for both practical and ethical issues, are urine and, to a lesser extent, blood. Biological exposure indexes (BEI) or limit values have been proposed by various bodies for certain pesticides or their metabolites, including parathion, pentachlorophenol, some organochlorines, 2,4-D, and MCPA (see relevant paragraphs). For other compounds, data are available but have not been validated and in most cases neither the metabolism of the active ingredient is completely known, nor is the relationship between urinary/blood levels of a compound/metabolite and the toxic effects understood. In particular, it should be remembered that metabolism may differ according to the route of absorption (Woollen *et al.*, 1992). The only biomarker of effects proposed by the ACGIH and other bodies (e.g. the UK HSE, the WHO) is the measurement of erythrocyte acetylcholinesterase, for which reference values have been determined (see pertinent paragraph also for a discussion on plasma cholinesterase).

Toxicological effects of occupational exposure to pesticides

This section describes the most relevant effects of occupational exposure to pesticides. When possible, an indication of the dose–response relationship will be given based on information obtained from non-occupational poisonings. Information on biological monitoring will also be given and its toxicological relevance discussed.

Table 11.1 reports the pesticides that caused either allergic contact dermatitis or other forms of dermatitis. These will not be discussed. Table 11.2 reports IARC evaluations of the carcinogenic potential of pesticides. Except for a few compounds, these data will also not be discussed here.

Table 11.1 Pesticides causing allergic contact dermatitis (ACD) or orthoergic dermatitis (OD) (summarized from Hayes and Laws, 1991, and Krieger, 2001, except where otherwise indicated)

	Type of dermatitis
Insecticides	
Organophosphorus compounds	
Dichlorvos	ACD
Naled	OD/ACD
Thiometon	ACD
Organochlorine compounds	
Aldrin	OD
DDT	OD[a]
Dicofol	ACD
Lindane and BHC	OD/ACD[b]
Pyrethrum	ACD
Herbicides	
2,4-D	OD[b]/ACD
2,4,5-T	OD[c]/ACD
Alachlor	ACD[d]
Allidochlor	ACD
Atrazine	ACD
Cyanazine	ACD
Dichlobenyl	OD
Diquat	OD
MCPA	OD
Metholachlor	ACD
Nitrofen	OD
Paraquat	OD
Phenmedipham	ACD
Propachlor	ACD
Propanyl	OD[e]
Propazine	OD[f]
Simazine	OD
Fungicides	
1-chlorodinitrobenzene	ACD
Benefin	ACD[g]
Benomyl	ACD
Captan	ACD
Captafol	OC/ACD
Chlortalonil	ACD
Dinocap	ACD
Fluazinam	ACD[h]
Imazalil	ACD
Mancozeb	ACD

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Table 11.1 (*continued*)

	Type of dermatitis
Maneb	OD/ACD
Pentachlorophenol	OD[c]
Thiophanate-methyl	OD/ACD
Thiram	ACD
Trifluralin	ACD[g]
Zineb	OD/ACD
Ziram	OD
Rodenticides	
ANTU	ACD
Solvents and fumigants	
1,3-dichloroprene	OD
Kerosene	OD
Metam-sodium	ACD
Tetralin	OD
Xylene	OD
Inorganic and organometallic pesticides	
Arsenic and its compounds	OD
Chromium (sodium dichromate)	OD/ACD
Copper (cupric sulphate)	OD
Zinc (zinc chloride)	OD
Miscellaneous pesticides	
Chlorfenson	ACD
Propargite	ACD

DDT, 1,1,1-trichloro-2,2-*bis*(4-chlorophenyl) ethane; 2-4D, 2,4-dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; ANTU, α-napthylthiourea.
[a]Due to solvents, mainly xylenes.
[b]Probably due to contaminants.
[c]Chloracne was also described but it was likely caused by the contaminant TCDD (2,3,7,8-tetrachlorodibenzp-*p*-dioxin).
[d]From Won, Ahn, and Kim (1993). Nail lesions also described.
[e]Also chloracne caused by impurities.
[f]Probably caused by intermediates in the synthesis.
[g]From Pentel, Andreozzi, and Marks (1994).
[h]From van Ginkel and Sabapathy (1995).

DDT and its analogues

Despite its widespread and sometimes careless use, the safety record of DDT is exceptionally good. Thousands of people have been dusted with DDT powder, workers have sprayed the compound in such a way that dermal and respiratory exposure was huge and yet no definite lethal poisoning has ever been reported. Mild effects such as headache, dizziness, and paresthesia of the extremities have been reported in heavily exposed workers (Smith, 2001). DDT is a known inducer of liver microsomal enzymes

Table 11.2 IARC evaluation of pesticides

Compound	Year	Degree of evidence for carcinogenicity[a]		Overall evaluation[b]
		Human	Animal	
Insecticides				
Agents and groups of agents				
Aldicarb	1991	ND	I	3
Aldrin	1987	I	L	3
Aramite	1974	ND	S	2B
Arsenic and arsenic compounds	1987	S	L	1[c]
Carbaryl	1976	ND	I	3
Chlordane/heptachlor	2001	I	S	2B
Chlordimeform	1983	ND	I	3
Chlorobenzilate	1983	ND	L	3
DDT	1991	I	S	2B
Deltamethrin	1991	ND	I	3
Dichlorvos	1991	I	S	2B
Dicofol	1983	ND	L	3
Dieldrin	1987	I	L	3
Endrin	1974	ND	I	3
Fenvalerate	1991	ND	I	3
Hexachlorocyclohexanes (HCH)	1987	I		2B
Technical-grade HCH			S	
α-HCH			S	
β-HCH			L	
γ-HCH (lindane)			L	
Malathion	1983	ND	I	3
Methoxychlor	1979	ND	I	3
Mirex	1979	ND	S	2B
Parathion	1983	ND	I	3
Parathion-methyl	1987	ND	ESL	3
Piperonyl butoxide	1983	ND	I	3
Tetrachlorvinphos	1983	ND	L	3
Toxaphene	2001	I	S	2B
Trichlorfon	1983	ND	I	3
Zectran[d]	1976	ND	I	3
Mixtures				
Terpene polychlorinates (Strobane)	1974	ND	L	3
Fungicides				
Captafol	1991	ND	S	2A
Captan	1983	ND	L	3

continues overleaf

Table 11.2 (*continued*)

Compound	Year	Degree of evidence for carcinogenicity[a]		Overall evaluation[b]
		Human	Animal	
Chlorophenols	1978	L		2B
Pentachlorophenol	1991	I	L	3
2,4,5-Trichlorophenol[e]			I	
2,4,6-Trichlorophenol[e]			S	
Chlorothalonil	1999	I	S	2B
Copper 8-Hydroxyquinoline	1977	ND	I	3
Ferbam	1976	ND	I	3
Maneb	1976	ND	I	3
o-Phenylphenol	1999	I	L	3
o-Phenylphenate	1999	I	S	2B
Quintozene (Pentachloronitro-benzene)	1974	ND	L	3
Sodium ortho-phenylphenate	1987	ND	S	2B
Thiram	1991	I	I	3
Zineb	1976	ND	I	3
Ziram	1991	ND	L	3
Herbicides				
Amitrole	2001	I	S	3
Atrazine	1991	I	S	2B
Chlorophenoxy herbicides	1987	L		2B
2,4-DI			I	
2,4,5-TI			I	
MCPA ND			ND	
Chloropropham	1976	ND	I	3
Diallate	1983	ND	L	3
Fluometuron	1983	ND	I	3
Monuron	1991	ND	L	3
Propham3	1976	ND	I	3
Picloram	1991	ND	L	3
Simazine	1991	ND	I	3
Sulfallate	1983	ND	S	2B
Trifluralin				
Other				
1,2-Dibromo-3-chloropropane	1999	I	S	2B
1,3-dichloropropene	1999	ND	S	2B
Dimethylcarbamoyl chloride[f]	1987	I	S	2A
Ethylene dibromide[g]	2001	I	S	2A

Table 11.2 (*continued*)

Compound	Year	Degree of evidence for carcinogenicity[a]		Overall evaluation[b]
		Human	Animal	
Hexachlorobenzene	2001	I	S	2B
Methyl bromide	1999	I	L	3
Naphthylthiourea (ANTU)[h]	1987	I	I	3

2-4D, 2,4-dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; DDT, 1,1,1-trichloro-2,2-*bis*(4-chlorophenyl) ethane; HCH, hexachlorocyclohexane; MCPA, 4-chloro-2-methylphenoxyacetic acid.

[a]I, inadequate evidence; S, sufficient evidence; L, limited evidence; ND, no data; ESL, evidence suggesting lack of carcinogenicity.

[b]1, the agent is carcinogenic to humans; 2A, the agent is probably carcinogenic to humans; 2B, the agent is possibly carcinogenic to humans; 3, the agent is not classifiable as to its carcinogenicity to humans; 4, the agent is probably not carcinogenic to humans.

[c]This evaluation applies to the group of chemicals as a whole and not necessarily to all individual chemicals within the group.

[d]Also a molluscide.

[e]Primarily used as chemical intermediates. Soil fumigant/nematocide.

[f]Pesticide intermediate.

[g]Soil fumigant.

[h]Rodenticide.

when given at high doses; but occupational exposures have rarely been reported to be associated with indication of liver enzyme induction (Smith, 2001), even in subjects with levels of DDT in fat about 2 orders of magnitude higher than those in the general population (Laws, Curley, and Bios, 1967).

Exposure to DDT has been associated with increased liver and breast cancer incidence but available data are either negative or inconclusive (FAO/WHO, 2001; Smith, 2001).

DDT analogues such as dicofol, chlorobenzilate, methoxychlor, ethylan, and TDE have been used to a lesser extent and cases of occupational poisoning are rare. Symptoms were non-specific and always very mild (Smith, 2001).

HCB and lindane

There are few reports of acute poisoning after occupational exposure to HCB or lindane (Smith, 1991). These were generally mild showing headache, tremors, vomiting, prostration, and sedation. Long-term effects were not demonstrated in exposed workers, except for some abnormal EEG patterns in one study in HCB exposed workers (Mueller *et al.*, 1981). Biological limit values of 25 and 20 μg lindane/l of plasma have been proposed by the German Authorities and the UK HSE, respectively (DFG, 2000; Wilson, 1999).

Organophosphorus compounds

Many reports are available on cases of organophosphate (OP) poisoning which are mostly but not solely due to suicide attempts (Karalliedde, Eddleston, and Murray, 2001). OP poisoning is relatively uncommon in Western countries whereas it represents a significant health problem in developing countries. Clinical signs of OP poisoning, due to excess of acetylcholine at nerve terminals caused by acetylcholinesterase (AChE) inhibition, are classified as muscarinic (salivation, sweating, lacrimation, bronchorrhea and bronchoconstriction, abdominal cramps and diarrhoea, miosis, and bradycardia), nicotinic (fasciculation with muscular weakness, tachycardia, hypertension), and central (confusion, anxiety, tremor, blurred vision, convulsions, respiratory depression, coma). The delay in onset of the signs after poisoning depends mainly on the route of exposure and the compound involved. Signs may occur within minutes after massive poisoning with a rapidly absorbed compound. In the case of slowly absorbed or slowly activated compounds the onset may occur a few hours later. Usually the muscarinic signs appear first and in various combinations. Severe, life-threatening poisonings are characterized by respiratory failure due to the combination of the described effects. Recovery time depends on the dose, on the metabolism of the OP involved, and the treatment. In fact, certain OPs are slowly eliminated and the compound could be found in plasma several days after poisoning. Recovery from acute OP poisoning usually is complete within few days, unless severe hypoxia or convulsions have occurred (Lotti, 2001). Diagnosis of OP poisoning is rather easily made on clinical grounds and measurement of AChE activity in erythrocyte (see below) should be used only for confirmation and treatment should not be delayed until this information is available. In addition, determination of erythrocyte AChE activity is not useful in assessing the course of the poisoning because its time-course of inhibition does not correlate with that of nervous system AChE (Lotti, 1991).

A further form of toxicity, called the intermediate syndrome, has also been described (Senanayake and Karalliedde, 1987). It is characterized by weakness or paralysis of extraocular, facial, respiratory, neck flexor, and proximal limb muscles, which is resistant to atropine and oxime therapy and often requires assisted ventilation. Symptoms appear 1–4 days after the onset of the cholinergic syndrome, with or without a symptom-free interval and recovery varies from a few days to a few weeks. The incidence of the intermediate syndrome varies from about 8 per cent (He *et al.*, 1998) to 40 per cent (De Bleecker *et al.*, 1992) of patients with severe cholinergic toxicity although a properly conducted prospective study is not yet available. De Bleecker (2001) concluded, on the basis of available data, that (a) the intermediate syndrome coincides with prolonged AChE inhibition usually associated with a prolonged toxicokinetics of the compound (high liposolubility and/or prolonged metabolism and excretion because of impaired cardiovascular, hepatic, or renal function); (b) the syndrome is not due to muscle necrosis; (c) the syndrome can be better explained by combined pre- and post-synaptic impairment of muscular transmission; and

(d) the syndrome bears no relation to organophoshate-induced delayed polyneuropathy (OPIDP). It has also been proposed that the intermediate syndrome might be an artefact of insufficient oxime therapy (Benson, Tolo, and McIntive, 1992).

Poisoning episodes due to occupational exposure are relatively rare and mostly occur in developing, tropical countries: in these areas poor hygienic conditions are often encountered because of extreme climatic conditions, lack of proper education and training of farmers, and insufficient resources for proper personal protection devices and maintenance of equipment (Agarwal, 1993; Karalliedde, Eddleston, and Murray, 2001). Improper storage of the compound may also cause the formation of more toxic degradation products leading to poisoning in normal use conditions (Baker *et al.*, 1978; Soliman *et al.*, 1982). Most frequently, poisonings occur in agricultural settings but cases have also been described in factories where OPs were being produced or formulated (Jones, 1982). Mild poisoning has also been described in health workers treating a contaminated patient (CDC, 2001).

Some OP insecticides have caused another form of toxicity called OPIDP. This is characterized by degeneration of distal parts of long- and large-diameter axons in peripheral nerves and also histopathological changes in the spinal cord (Lotti, 2001). The onset of clinical symptoms occurs 2–4 weeks after poisoning and is characterized by cramping muscle pain in the legs rapidly followed by distal paresthesias and leg weakness with reduced deep tendon reflexes. In severe cases involvement of upper limbs also occurs and quadriplegia may develop. In this case, pyramidal signs also appear because of spinal cord involvement. There might be some functional recovery of peripheral nerves within months from onset; in this case pyramidal signs became more evident and ultimately determine the evolution towards spastic ataxia. In general, sensory involvement is mild. Electrophysiological studies reveal reduced amplitude of compound muscle action potentials and delayed terminal latencies after supramaximal stimulation of motor nerves: maximal conduction velocity is generally normal or slightly reduced (Lotti, Becker, and Aminoff, 1984). In severe cases, these parameters might not be measurable. Electromyography reveals denervation of affected muscles (fibrillation potentials and positive sharp waves) and reduced interference pattern. Convincing evidence of OPIDP based on clinical and, sometimes, analytical data and supported by experimental information is available for chlorpyrifos (Lotti *et al.*, 1986), dichlorvos (Vasilescu and Florescu, 1980; Wadia, Shinde, and Vaidya, 1985), isofenphos (Moretto and Lotti, 2002), methamidophos (Senanayake and Johnson, 1982), trichlorfon (Vasilescu, Alexianu, and Dan, 1984), and trichloronat (Jedrzejowska, Rowiska-Marciska, and Hoppe, 1980). Except in the case of methamidophos, all cases of OPIDP followed suicide attempts. Some of the more than 20 cases of OPIDP caused by methamidophos occurred in farm workers. In these cases, poor hygienic conditions and lack of use of protective equipment were identified (Senanayake and Johnson, 1982). OPIDP development occurred 2–4 weeks after poisoning and, as expected from experimental data, it was preceded by a severe cholinergic syndrome which required prolonged treatment with atropine, oximes

and, frequently, assisted ventilation. One exception is the case of isofenphos poisoning described by Moretto and Lotti (2002): the patient developed a severe OPIDP about 3 weeks after poisoning but the acute cholinergic syndrome was very mild. However, the patient was treated with very high doses of atropine and oxime for about 2 weeks. This is in contrast with experimental data in hens which show that the neuropathic dose of isofenphos always elicits a severe cholinergic syndrome with high mortality (Wilson *et al.*, 1984). The likely explanation for this discrepancy is the prompt and continuous (infusion) treatment with atropine and 2-PAM provided to the patient for several days. Infusion is required because atropine and 2-PAM have a short half-life in humans (Adams *et al.*, 1982; Jovanovic, 1989; Kanto and Klotz, 1988; Sidell, Groff, and Kaminskis, 1972; Willems *et al.*, 1992). Such a treatment usually is not provided nor can it be easily done in hens. Therefore, the doses of isofenphos which can be administered to hens would be much lower than those to which humans will survive, if properly treated. Since this may also occur with other OPs, it must be kept in mind when performing risk assessment for OPIDP.

In patients who survived acute OP poisoning, several late psychiatric, neurobehavioural, and neurological abnormalities have been described. One author grouped these abnormalities together in a syndrome called chronic OP-induced neuropsychiatric disorders (COPIND phenomenon I) (Jamal, 1997). Abnormalities such as retrograde amnesia, cerebellar ataxia, EEG changes, increased vibrotactile threshold, dizziness, sleepiness, headache, and deficits in a number of neuropsychological and psychomotor tests have been described. However, prospective studies have never been performed and available reports lack details or information, or have a faulty design preventing meaningful conclusions (summarized in Lotti, 2001; Ray, 1998a). These authors concluded that there is little evidence that, in the absence of hypoxia and/or convulsions in the early phase, acute OP poisoning results in late permanent neurological or psychiatric effects other than OPIDP. In addition, the inclusion of all these different effects into a single syndrome such as COPIND was considered inappropriate and possibly misleading (Lotti, 2001).

Long-term exposure to a low level of OPs was also associated with a number of psychiatric, neurobehavioural, and neurological effects which have been discussed in several recent reviews (Brown and Brix, 1998; ECETOC, 1998; Lotti, 2001; Ray, 1998a, 1998b; Steenland, 1996). Jamal (1997) grouped all this effects under the syndrome called COPIDN phenomenon 2 and this approach again has been criticized (Lotti, 2001). Most of the studies where neurobehavioural tests were performed gave negative results except when the subjects were still exposed with some evidence of AChE inhibition (Gomes *et al.*, 1998). Studies were conducted to identify an association between OP exposure and suicide rates or psychiatric disorders. These were either negative (Pickett *et al.*, 1998; Stoller *et al.*, 1965) or inconclusive due to lack of information on either actual exposure or confounding factors (Amr, Halim, and Moussa, 1997; Levin, Rodnitzky, and Mick, 1976; Parrón, Hernández, and Villanueva, 1996). Neurological effects on the central and peripheral

nervous system described in OP-exposed individuals include the visual syndrome known as Saku disease, minor EEG disturbances, sensory neuropathies or deficit in some sensory tests, and electrophysiological disturbances of the neuromuscular function. Saku disease (reduced visual field, myopia, astigmatism, lesions of the optic nerve, abnormal retinal functions) occurred in the 1960s in selected areas of Japan and the aetiological link with OP exposure was questioned (Dementi, 1994; Pleština and Piuković-Pleština, 1978). Studies on neuromuscular and sensory function have already been reviewed by several authors (ECETOC, 1998; Lotti, 2001; Ray, 1998b) and the conclusion was that there is no indication of an association between low level exposure to OPs and effects on the nervous system.

The metabolism of OP compounds is variable because some may undergo extensive degradation whereas there are some instances where the compound is mainly excreted unchanged. The most common hydrolytic pathway is the breakage of the P-ester bond giving the alcoholic moiety ('leaving group') and the acidic moiety (alkyl(thio)phosphates). Dimethylated OPs give dimethylphosphates, dimethylthiophosphates, dimethyldithiophosphates, and dimethylphosphorothioates, whereas diethylated OPs give the corresponding diethylated metabolites (Coye, Lowe, and Maddy, 1986; IPCS, 1986a). Since they derive from a large number of compounds, alkylphosphates are non-specific metabolites. Therefore, the compound to which the worker is exposed must be known if a toxicological significance is to be given to the data. For instance, dimethylated OPs have a wide range of acute toxicity, and yet they may give the same amount of dimethylphosphates; the same applies to diethylated OPs. Measurement of urinary excretion of the alcoholic moiety would be more specific, but it is less frequently used. These include 3,5,6-trichloro-2-pyridinol after exposure to chlorpyrifos-ethyl and chlorpyrifos-methyl (Nolan *et al.*, 1984), malathion mono- and dicarboxylic acids after exposure to malathion (Bradway and Shafik, 1977), and *p*-nitrophenol after exposure to parathion (Morgan *et al.*, 1977). Differences in toxicokinetics between OPs or according to the exposure route further complicate the issue. Therefore the timing of urine sampling is critical and has to be chosen according to the characteristics of the compound and of the exposure. There is a large number of field studies in which exposure was assessed by means of determination of urinary metabolites but most of the data on excretion of metabolites are not toxicologically relevant since little information is available about the correlation between the amount of urinary metabolites and inhibition of blood enzymes (erythrocyte AChE and/or plasma cholinesterase, see below). In fact, in most of these field studies erythrocyte AChE was found either not or only minimally inhibited (Aprea *et al.*, 1997; Griffin *et al.*, 1999; Jauhiainen *et al.*, 1992; Kraus *et al.*, 1977; Krieger and Thongsinthusak, 1993; Maroni *et al.*, 1990; Popendorf *et al.*, 1979; Spear *et al.*, 1977). The only fairly good correlation found was that between urinary *p*-nitrophenol excretion and erythrocyte AChE inhibition in workers exposed to parathion (Arterberry *et al.*, 1961). This study supports the BEI established by the ACGIH of 0.5 mg of *p*-nitrophenol/g of creatinine (ACGIH, 2001). Moreover, estimation of the absorbed

dose from plasma/urinary levels of the compound or its metabolite(s) is rarely possible due to a lack of toxicokinetic information in humans. Attempts have been made to correlate urinary excretion of alkylphosphates with the no observed adverse effect level (NOAEL) after exposure to azinphos-methyl using a toxicokinetic model based on both animal and human data (Carrier and Brunet, 1999).

In conclusion, the determination of plasma or urinary levels of the parent compound or its metabolite(s) is certainly a sensitive method to monitor occupational exposures, given the sophisticated analytical techniques available. However, these data can only be considered qualitative indexes of exposure rather than toxicologically significant values.

Since AChE is also present in the outer membrane of the erythrocytes, measurements of its activity can be used to monitor occupational exposures to OPs (IPCS, 1986a; Jeyaratnam and Maroni, 1994; Pleština, 1984; Wilson, Jaeger, and Baetcke, 1992). The first clinical signs are observed when 50 per cent of AChE is inhibited in the nervous system; severe symptoms and death occur at >80 per cent and >90 per cent inhibition, respectively, if treatment is not provided. However, the ratio between AChE inhibition in erythrocytes and in nervous tissue is not necessarily equal to 1. Data from experimental animals indicate that there are significant differences in the accessibility of the nervous system to compounds; therefore, although compounds will always have better access to erythrocytes the difference might be trivial for those compounds that readily cross the blood–brain barrier but relevant for those which do not. In addition, it should be remembered that the blood–brain barrier is more efficient than the blood–nerve barrier. Consequently, erythrocyte AChE inhibition usually over-estimates that in the nervous tissue, but the extent of this over-estimation is generally not known. Other problems that need to be taken into account when interpreting erythrocyte AChE data are: (a) the high interindividual and the somewhat lower intraindividual variability: for these reasons only reductions >20 per cent on normal average values or of 10–20 per cent on individual pre-exposure value(s) can be considered significant (Lotti, 2001); (b) inhibition by dimethylphosphates can be partly reversible and, therefore, this must be taken into account if measurement is not performed immediately after exposure; (c) the rate of reappearance of AChE activity due to resynthesis of the enzyme is quicker in the nervous tissue than in erythrocytes: animal data indicate that the half-life in the nervous system is 5–7 days (Lotti, 1992) whereas that in blood depends on new erythrocytes produced by the bone marrow at an estimated rate of 1 per cent per day (Lotti, 2001; Mason, 2000). According to values found, guidelines for intervention in occupational settings have been proposed (Jeyaratnam and Maroni, 1994): inhibitions up to 30 per cent and up to 60 per cent against pre-exposure values or up to 50 per cent and up to 70 per cent against normal reference values require medical surveillance plus examination of working conditions, and temporary removal from exposure plus modifications of working conditions, respectively. ACGIH (2001) has established a biological exposure index at 70 per cent of individual baseline. Various criteria have been produced for workers' readmission to

work: Pleština (1984) suggested waiting until erythrocyte AChE activity values are back to normal; in the United Kingdom, the Health and Safety Executive (HSE, 1987) leaves the decision to the physician; while the California Environmental Protection Agency requires 80 per cent of baseline activity (Wilson *et al.*, 1997).

Plasma pseudocholinesterase activity, an enzyme with no known physiological substrate, can also be inhibited by OPs but this inhibition has no toxic correlate (Lotti, 2001). The sensitivity of pseudocholinesterase to inhibition differs from that of AChE and the *in vivo* ratios between the sensitivity of these two enzymes varies according to the timing of sampling after exposure and the exposure pattern (single versus repeated). In humans, dimefox, mevinphos, parathion, and parathion-methyl preferentially inhibit erythrocyte AchE, whereas chlorfenvinphos, chlopyrifos, demeton, diazinon, dichlorvos, fenitrothion, malathion, monocrotophos, and trichlorfon preferentially inhibit pseudocholinesterase activity (Moretto and Lotti, 2001). As a consequence, in the absence of data on erythrocyte AChE, pseudocholinesterase activity can only be considered a marker of exposure rather than effect. The (lack of) inhibition of AChE might be extrapolated when the ratio between the sensitivity to inhibition of these two enzymes is known.

Neuropathy target esterase (NTE), the putative target of OPIDP, is present in circulating lymphocytes in addition to nervous tissue (Bertoncin *et al.*, 1985). Measurement of lymphocytic NTE has been proposed for monitoring exposure to neuropathic OPs (Lotti, 1987), but its large interindividual variability, the different access of the OPs to the nervous system, the different rates of resynthesis between the nervous system and lymphocytes limit its usefulness. In fact, it has been only been employed as a prognostic test for the development of OPIDP (Moretto and Lotti, 1998).

Carbamates

Carbamates cause comparatively fewer cases of occupational, accidental, and voluntary poisonings than OPs. Occupational cases have been described with carbofuran (Smith and Lewis, 1988; Tobin, 1970), mexacarbate (reported by Ecobichon, 2001), carbaryl (IPCS, 1994a), propoxur (Sidhu and Collisi, 1989), aldicarb (IPCS, 1991; Ragoucy-Sengler *et al.*, 2000) and methomyl (Cable and Doherty, 1999; IPCS, 1996). Most of these cases were relatively mild and rapidly reversible. In general, carbamates are considered less toxic than OPs; however, cases of acute severe and fatal poisoning by certain carbamates are described in the literature (Ecobichon, 2001; IPCS, 1986b). Signs and symptoms of the carbamate induced cholinergic syndrome are those described for OPs, the main difference being their shorter duration.

Carbamates usually undergo extensive metabolism, especially by carboxylesterases forming an aryl alcohol plus a methyl- or dimethyl-carbamic acid. The latter will rapidly decompose into carbon dioxide and mono- or dimethyl-amine. However, the rate of metabolism is dependent on the structure of the individual compound. Side-chains may also undergo oxidation (e.g. hydroxymethylation),

N-demethylation of secondary amines attached to the aryl moiety, or ring hydroxylation via the formation of an epoxide intermediate. Thiocarbamates (e.g. aldicarb) may undergo *S*-oxydation forming the correspondent sulphone and sulphoxide. Urinary metabolites are mainly represented by glucuronide or sulphate derivatives of the aryl groups. The parent compound may be found in small amounts in the urine (IPCS, 1986b).

Determinations of urinary excretion of metabolites such as α-naphthol from carbaryl (see IPCS, 1986b, and references therein), 3-hydroxycarbofuran, and 3-ketocarbofuran from carbofuran (Huang *et al.*, 1989), 2-(di)methylamino-4-hydroxy-5,6-dimethylpyrimidine and other hydroxypyrimidines from pyrimicarb (Hardt, Apll, and Angerer, 1999; Verberk *et al.*, 1990), and 2-isopropoxyphenol from propoxur (Brouwer *et al.*, 1993) exposures have been carried out but no correlation could be established with acute effects on blood cholinesterases.

Measurements of carbamate-induced AChE inhibition are hampered by the relatively rapidly reversible inhibition they cause. Care should be taken to reduce the time between blood sampling and assay; blood should be kept cold, and the sample dilution during assay should be minimal. Moreover, the time of assay and the substrate concentration should also be kept to a minimum (Lotti, 1991). Other limitations on the measurement of erythrocyte AChE have been described in the paragraph discussing OPs.

Synthetic pyrethroids

Occupational exposure to synthetic pyrethroids, when associated with deposition of the pesticide on the skin, causes transient sensory facial symptoms. These generally are described as burning sensations, tingling, itching, tightness, or numbness. The face, usually the periorbicular area, forehead, and cheeks, is most frequently affected (Chen *et al.*, 1991; He *et al.*, 1988; 1991; Kolmodin-Hedman, Swensson, and Akerblom, 1982; Le Quesne, Maxwell, and Butterworth, 1980; Moretto, 1991; Tucker and Flannigan, 1983; Zhang *et al.*, 1991). Apparently all types of synthetic pyrethroids cause these effects, although with different potency. In general, 30–70 per cent of the workers complain of these abnormal cutaneous sensations. The symptoms occur within a few hours from the beginning of the exposure and spontaneously resolve within 24 h. These symptoms can be rather annoying but not disabling and are not associated with adverse effects on peripheral nerve conduction (Le Quesne, Maxwell, and Butterworth, 1980). It is believed that these symptoms are due to the interaction of the synthetic pyrethroid with the sodium channels of the sensory nerve terminals in the skin causing spontaneous repetitive firing, although the mechanism has never been studied in detail. Symptoms can be alleviated by rubbing the skin with oil (Malley *et al.*, 1985) whereas water and soap are ineffective (Moretto, 1991). Other local, transient effects include rhinorrhea, nose and throat irritation with sneezing and coughing.

Systemic signs and symptoms are unlikely to occur during occupational exposure to synthetic pyrethroids if good work practices and safety precautions are observed. However, a number of cases of acute synthetic pyrethroid poisoning have been described (Chen *et al.*, 1991; He *et al.*, 1988, 1989) following exposure in poor hygienic conditions. These were characterized by headache and nausea, and in more severe cases, muscle fasciculation and convulsive attacks. Followed-up cases did not show detectable permanent sequelae (He *et al.*, 1989).

Urinary excretion of synthetic pyrethroids and/or their metabolites was studied in several groups of exposed workers. However, no correlation was found between extent and duration of skin symptoms and urinary excretion of fenvalerate or deltamethrin and their metabolites (Chen *et al.*, 1991; He *et al.*, 1988, 1991; Kolmodin-Hedman, Swensson, and Akerblom, 1982; Zhang *et al.*, 1991). In other studies, no signs were reported in subjects with detectable levels of the active ingredient or its metabolite(s) following occupational or experimental exposure to different synthetic pyrethroids (Angerer and Ritter 1997; Aprea, Stridori, and Sciarra, 1997; Eadsforth and Baldwin, 1983; Eadsforth, Bragt, and van Sittert, 1988; Leng, Kuhn, and Idel, 1997; Leng *et al.*, 1997a, 1997b; Llewellyn *et al.*, 1996; Wilkes *et al.*, 1993; Woollen *et al.*, 1992).

Paraquat

Most of the information on paraquat toxicity in humans derives from cases of acute poisoning due to suicidal or, much less frequently, to accidental ingestion of paraquat. Accidental poisonings in occupational settings generally occurred because of storage of the formulation in unlabelled bottles or other containers. Unlike suicide attempts, these cases are now decreasing, in part because the manufacturer of paraquat changed the commercial formulations by adding a stenching agent to alert users, a blue colour to avoid confusion with drinks, and an emetic. Improved education and training have also concurred to this result (Lock and Wilks, 2001a; Wesseling *et al.*, 1997b). In the most severe poisonings, liver and kidney necrosis are most prominent and death occurs within few days because of multiorgan failure. This usually occurs at doses above 50 mg/kg. Less acutely severe cases (doses from about 20 to 50 mg/kg) are characterized by recovery from liver and kidney injury followed by lung fibrosis leading to respiratory failure which is frequently fatal within one to several weeks (Lock and Wilks, 2001a). Lung fibrosis is due to accumulation of paraquat in the lungs because of a specific energy-dependent uptake system (details on the mechanism are given in the specific chapter). Ingestion of paraquat is generally associated with ulceration in the throat, oesophagus, and stomach with risk of perforation. There are indications that survivors of severe paraquat poisoning may develop a relatively mild restrictive pulmonary dysfunction (Bismuth *et al.*, 1996). Follow-up of the patients was not long enough (2–6 years) to determine whether this dysfunction was progressive

(Yamashita, Yamashita, and Ando, 2000). In two cases, improvement of lung function occurred over 4–10 years (Bismuth *et al.*, 1996).

Occupational exposure to paraquat caused topical effects mainly during spray operations with poor working practice and hygienic behaviour (Howard, 1979). Usually caustic lesions occurred on the skin, in mucosae, or the eye; when measured, little or no absorption of the compound was found, as determined by paraquat levels in urine (Hoffer and Taitelman, 1989; Swan, 1969; van Wendel de Joode *et al.*, 1996). Typically the skin appears pale, then blistering, and ulcerations might occur. The latter require contact with the concentrated formulation or prolonged contact with the diluted spray preparation (Hearn and Keir, 1971; Howard, 1979). Nail damage was also observed, which included loss of nail surface, transverse ridging, gross deformity, or loss of the nail (Hearn and Keir, 1971). These lesions recovered after cessation of exposure. A number of cases of eye damage resulting from splashes usually with the concentrate formulation of paraquat have been described. In these cases recovery was complete (Lock and Wilks, 2001a). Lesions included irritation and blepharitis, destruction of areas of the conjuntiva or of the corneal epithelium.

Few severe, and even fewer fatal, cases of occupational paraquat poisoning have been reported following excessive skin contact. In these cases paraquat was not used as recommended and a combination of factors such as incorrect dilution, use of faulty equipment, prolonged and extensive skin contact because of lack of appropriate protective equipment, disregard of safety procedures (including decontamination after skin contact), and previous skin damage led to excessive absorption of paraquat (Smith, 1988; Wesseling *et al.*, 1997b). In all cases there were severe paraquat-induced skin lesions including blistering, burning, or ulceration. Concentrations as low as 5 g/L were found to be effective if dermal exposure was extensive and prolonged (Athanaselis *et al.*, 1983), although upon more detailed investigation, the concentration in the case described was found to have been higher than 15 g/L (Hart, 1984). Otherwise, owing to the low skin penetration of paraquat (Wester *et al.*, 1984), no significant absorption is to be expected from normal occupational use of paraquat. This is confirmed by field studies where ambient and biological monitoring was carried out (Chester and Ward, 1984; Chester and Woollen, 1981; Staiff *et al.*, 1975; Swan, 1969; Wojeck *et al.*, 1983).

Paraquat has an extremely low vapour pressure and spray application generates droplets too large to enter the respiratory tract beyond the nasal cavity. In fact, only one case of mild poisoning (slight, rapidly reversible alterations of clinical–chemical tests of kidney function) following inhalation of paraquat has been reported (Fitzgerald *et al.*, 1978).

No adverse effects on lung function were observed in workers repeatedly exposed to paraquat (Castro-Gutierrez *et al.*, 1997; Howard, Sabapathy, and Whitehead, 1981; Senanayake *et al.*, 1993). More recently, Dalvie *et al.* (1999) studied lung function in workers exposed to paraquat. Exposure was assessed by workers' interview and expert opinion was used to weigh life-time cumulative exposures

based on duration and type of exposure. Among all tests performed, only one was affected, and that only slightly, namely exercise oxymetry. However, upon statistical analysis of the data, long-term paraquat exposure accounted only for a small proportion of the variance of the data, which renders the interpretation of the data difficult and their toxicological significance uncertain.

Given the apparent structural similarity between paraquat and 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) which caused a Parkinson-like syndrome in young drug abusers (Langston *et al.*, 1983), the hypothesis was made of an association between paraquat exposure and increased incidence of Parkinson's disease (Sanchez-Ramos, Hefti, and Weiner, 1987). A number of epidemiological studies have been carried out among agricultural workers and people living in rural areas, with inconsistent results. Some studies pointed to an association between the use of herbicides in general (Globe, Farrell, and Davis, 1990; Ho, Woo, and Lee, 1989; Semchuck, Love, and Lee, 1991) or paraquat in particular (Hertzman *et al.*, 1990; Liou *et al.*, 1997) and increased risk of Parkinson's disease. Other studies could not demonstrate such an association (Koller *et al.*, 1990; Ohlson and Hogstedt, 1981; Tanner, Grabler, and Goetz, 1988; Tanner *et al.*, 1990; Zayed *et al.*, 1990). However, it should be noted that the similarity between paraquat and MPTP is only apparent, because their biological behaviour is different. In particular, paraquat is not significantly metabolized and, being a di-cation, it does not cross the blood–brain barrier to a significant extent (Naylor *et al.*, 1995; Widdowson *et al.*, 1996a, 1996b). On the other hand, there are some indications that intraperitoneal treatment with paraquat causes dopaminergic neuron loss in mice, similar to that induced by MPTP (Brooks *et al.*, 1999). It should be noted that, even in the most severe poisonings by paraquat, specific effects on the nervous system were never reported nor have neurological problems been recorded in workers repeatedly exposed to paraquat. Follow-up of 12 survivors from paraquat poisoning did not show Parkinsonian symptoms (Yamashita, Yamashita, and Ando, 2000).

Paraquat is excreted unchanged in urine and therefore urinary paraquat excretion is a very good and reliable tool for biological monitoring. Several studies have been performed in exposed workers and levels of urinary paraquat were either below detection limits or far from those that correlated with signs of systemic poisoning (Chester and Woollen, 1981; Swan, 1969; Wojeck *et al.*, 1983). While there are no established biological limits for plasma or urinary paraquat, a relationship between plasma paraquat levels and survival could be obtained from acute poisoning data (Hart, Nevitt, and Whitehead, 1984; see also Lock and Wilks, 2001a for an extended review).

Diquat

Occupational exposure to diquat sometimes resulted in topical effects such as epistaxis after splashing, and nail damage after contact with the concentrate solu-

tion. Skin damage was also reported after prolonged contact with the spray solution. Lesions fully recovered.

Acute poisonings, which were mostly suicide attempts, resulted in liver and kidney damage and, when severe, neurological complications because of brain infarction or intracranial haemorrhage, and acute pulmonary oedema. Death usually is due to multiorgan failure and occurs at doses of about 200 mg/kg or above (Lock and Wilks, 2001b).

The measurement of urinary diquat can be used for biological monitoring of exposure. Urinary levels of diquat in exposed workers varied from undetectable to about 0.2 mg/L (Lock and Wilks, 2001b).

Pentachlorophenol

Several cases of occupational and non-occupational poisoning after acute or short-term exposure to pentachlorophenol (PCP) have been described. In most cases there was significant skin exposure and/or inhalation of PCP vapours in inadequate hygienic conditions. Signs and symptoms resemble those caused by nitrophenolic compounds because of a marked increase in metabolic rate due to the uncoupling of oxidative phosphorylation; these are characterized by sweating, tachycardia, tachypnea, hyperpyrexia, generalized weakness, nausea, vomiting, headache, and death. Certain kidney and liver function parameters may be altered and metabolic acidosis with anion gap has also been observed (Wood *et al.*, 1983).

There are anedoctal case reports that associate prolonged exposure to PCP with haemotoxicity (aplastic anaemia) (Roberts, 1983; Rugman and Cosstick, 1990) and miscarriages (de Mayer *et al.*, 1995). There are also reports of immunotoxic effects (mainly depressed T-cell activation) of exposure to PCP with apparently no significant clinical consequence (Colosio *et al.*, 1993, Daniel *et al.*, 1995; McConnachie and Zahalsky, 1991). Nerve conduction velocity was measured in 10 workers and found to be in the low to normal range (Triebig *et al.*, 1987) and, more recently, Peper, Ertl, and Gerhard (1999) found some deficits in neurobehavioural tests in women exposed to PCP as a result of living in houses where the compound was used as a wood preserver. Endocrine and reproductive function were also studied in subjects exposed to pentacholorophenol but no significant effects were found (Dimich-Ward *et al.*, 1996; Gerhard *et al.*, 1999).

Chronic exposure to PCP or to occupations involving a possible exposure to PCP were suspected of being associated with an increased incidence of non-Hodgkin's lymphomas, multiple myelomas, or soft tissues sarcomas (Eriksson, Hardell, and Adami, 1990; Hardell, Eriksson, and Degerman, 1994; Pearce *et al.*, 1986, 1987; Smith *et al.*, 1984). Results were mostly negative and, in any case, it was difficult to assess whether exposure to other compounds (such as dioxins and impurities of PCP) occurred.

Monitoring of exposure to PCP can be done by measuring plasma levels or urinary excretion of the unchanged compound: ACGIH (2001) proposes a biologi-

cal exposure index of 2 mg/g of creatinine (prior to the last shift of the work week) or 5 mg/L blood (end-of-shift).

Ethylene-*bis*-dithiocarbamates

Occupational exposure to ethylene-*bis*-dithiocarbamates (EBDCs) caused allergic contact dermatitis in few workers. Thyroid function was also studied in workers exposed to EDBCs or to their most active metabolite (ethylene thiourea [ETU]) and was found to be normal (Smith, 1984). The IARC also concluded that from available epidemiological data there was no indication that exposure to the EDBC degradation/metabolism product ETU caused an increased incidence of thyroid tumours (IARC, 2001).

The metabolic pathway of EBDCs is rather complex; however, ETU and carbon disulphide (CS_2) represent major metabolites. Mean urinary excretion of ETU in workers exposed to mancozeb or maneb was 2–4 mg/24 h (Kurttio and Savolainen, 1990). Based on studies in alcoholic patients given disulfiram, measurement of CS_2 levels in blood might be a useful tool for biological monitoring of exposure (Maranelli *et al.*, 1991).

Glyphosate

Accidental occupational exposures to glyphosate have been reported and only minor irritant effects on the eye were described (Acquavella *et al.*, 1999). Accidental ingestion generally resulted in minor mouth discomfort (Talbot *et al.*, 1991).

Several reports are available describing suicide attempts with glyphosate surfactant (Chang *et al.*, 1999; Menkes, Temple, and Edwards, 1991; Talbot *et al.*, 1991; Tominack, 1991). These are characterized by erosion of the gastro-intestinal tract (including the oesophagus) with sore throat, dysphagia, and gastro-intestinal haemorrhage. The lesions of the gastro-intestinal tract are associated with massive fluid and electrolyte loss which may also trigger acute renal tubular necrosis. Severe cases are also characterized by respiratory failure and pronounced hypotension which may be followed by seizures and death. Death occurs within 48 h of ingestion of more than 85 ml of the 41 per cent commercial formulation (i.e. about 0.5 mg glyphosate/kg bw) (Talbot *et al.*, 1991). It has been suggested that the surfactant may also play a role in the clinical picture (Sawada *et al.*, 1988). Glyphosate-trimesium has been reported to be more acutely toxic than glyphosate, apparently because of a much more rapid absorption (Sorensen and Gregersen, 1999).

Exposure to glyphosate can be monitored by measuring urinary excretion of the parent compound. However, in most of the exposed workers, levels of glyphosate were below the limit of detection (Farmer, 2001; Jauhiainen *et al.*, 1991; Lavy *et al.*, 1992).

Rodenticides

Fluoroacetic acid and its derivatives

Sodium fluoroacetate, fluoroacetamide, and fluoroethanol cause toxicity by blocking the Krebs cycle, leading to lowered energy production, reduced oxygen consumption, and reduced cellular concentration of ATP (Pelfrene, 2001).

The usage records of these compounds indicate that if they are used as directed, poisoning does not occur. Apparently only one case of sodium fluoroacetate poisoning has been described as work-related. However, the clinical and toxicological evidence of exposure was weak. Three cases of fluoroethanol poisoning have been reported in chemical workers as a consequence of accidents. All patients recovered within few days from tremor, severe muscle weakness, nausea, and headache (cited in Pelfrene, 2001).

A number of non-occupational accidental or suicidal poisoning cases are reported in the literature. The most recent refer to cases in Taiwan with a mortality rate of 18 per cent (Chi *et al.*, 1996). The clinical picture is characterized by metabolic acidosis with alteration in ECG (e.g. prolonged QT interval, altered T waves), hypocalcaemia, and hypokaliemia. The estimated lethal dose in humans for sodium fluoroacetate ranges from 2 to 10 mg/kg.

Anticoagulants

These compounds are anti-vitamin K agents thereby decreasing the synthesis of coagulation factors which are vitamin K-dependent (II, VII, IX and X). Some of these compounds, such as warfarin, also have therapeutic uses in humans.

Very few cases of poisoning by anticoagulants have been described in an occupational setting, usually derived from improper use of the compound (Pelfrene, 2001). These are characterized by reduced clotting power of the blood leading to bleeding after minimal trauma. The number of accidental or suicidal poisonings is higher and those due to the so-called ‘second generation’ anticoagulants or ‘superwarfarins’ (brodifacoum, chlorophacinone, difenacoum) appear to be more severe because of the long half-life of these compounds (Routh *et al.*, 1991). Developmental effects have been reported after use of anticoagulants (mainly warfarin) during pregnancy (Hall, Pauli, and Wilson, 1980; Schardein, 1985). However, no such effects have ever been described for anticoagulants used as rodenticides.

Determination of blood coagulation parameters, such as prothrombin time, can be used to monitor occupational exposure to anticoagulant rodenticides.

Fumigants

A number of fumigant pesticides have been withdrawn from the market or their use greatly reduced owing to their excessive toxicity; these include carbon disulphide, carbon tetrachloride, 1,2-dibromo-3-chloropropane, ethylene dibromide, and ethy-

lene dichloride (Burgess *et al.*, 2000). However, fumigant-related poisonings are still reported in the literature. The most frequently involved compounds are methyl bromide, phosphine, and 1,3-dichloropropene.

Methyl bromide

Exposure to methyl bromide occurs by inhalation, the dermal and oral route being of minor relevance. In many countries the use of methyl bromide is restricted to trained and licensed personnel. Many cases of either occupational or accidental poisoning are reported in the literature. Most poisoning incidents have involved unauthorized entry into buildings or persons living near buildings, greenhouses, or fields being fumigated with methyl bromide.

Methyl bromide in low concentrations is odourless so that a toxic atmosphere may not be apparent to the worker. Therefore, usually it is marketed in the form of 98 per cent methyl bromide and 2 per cent chloropicrin, as a lacrimatory agent. It is important to note that the manifestations of methyl bromide poisoning may be delayed from a few hours up to 48 h (IPCS, 1995).

Locally, methyl bromide is an intense vesicant on human skin. The acute and long-term effects of methyl bromide can be divided broadly into neurological and non-neurological. The principal non-neurological symptoms reported after acute inhalation of methyl bromide are associated with the respiratory system (chest pain or difficulty in breathing). The clinical picture in non-fatal poisoning is extremely variable. Fatigue, blurred or double vision, nausea, and vomiting are frequent; uncoordination, tremors, convulsions, exaggeration of the patellar reflexes, and a positive Babinski's sign may develop. In fatal poisoning, the early symptoms and signs are headache, visual disturbances, nausea and vomiting, smarting of the eyes, itching of the skin, listlessness, vertigo, and tremor. Progression is usually rapid, with the development of convulsions, neuropsychiatric signs and symptoms, such as mental confusion, mania, muscular twitches, and slurring of speech. Fever may occur as may tachypnoea, associated with pulmonary oedema, which may lead to death (Deschamps and Turpin, 1996; IPCS, 1995). The data pertaining to whether there is a correlation between blood bromide levels and the symptoms of methyl bromide poisoning are inconsistent. Some authors have suggested a direct correlation between blood bromide levels and the degree of intoxication (Hine, 1969; Rathus and Landy, 1961): 400 mg/L (ppm) – severe disability and death in some cases; 250 mg/L (ppm) – convulsive seizure and sometimes death; 176 mg/L (ppm) – slight residual ataxia; 135 mg/L (ppm) – moderate disability; 100 mg/L (ppm) or less – recovery, but symptoms of poisoning have been reported at bromide levels as low as 28 mg/L blood and severe symptoms at blood bromide levels of 120 mg/L (Rathus and Landy, 1961). Brenner (1978) and Bowers and Onoreski (1990) considered that the toxic threshold level for bromide in serum in humans was 500 mg/kg, although effects were observed in patients with lower levels.

Kishi *et al.* (1988) measured methyl bromide concentrations in the air in the workers' breathing zone, which were normally less than 4 mg/m^3, but, during some

accidental events, they exceeded 20 mg/m^3. The mean bromide ion concentration in the urine of men working in the manufacture of methyl bromide was 18.9 mg/L ± 10.4 (range 3.2–54.0 mg/L), with no correlation with the symptoms reported in a questionnaire. Other authors investigated whether there is a correlation between methyl bromide exposure levels and urinary bromide ion excretion, but results are conflicting (IPCS, 1995).

1,3-Dichloropropene

The most likely routes of human exposure to 1,3-dichloropropene are through inhalation and the skin. Exposure to 1,3-dichlopropene caused skin lesions due to its irritant potential (oedema, erythema, necrosis) and possibly contact dermatitis (see Table 11.1). Irritation of the eyes and upper respiratory mucosae, accompanied by lacrimation, also appear after exposure to vapours (Stott and Gollapudi, 2001). Several studies have been carried out in production or fumigation workers to assess whether 1,3-dichlopropene exposure had effects on sex hormone levels and sperm characteristics (Venable *et al.*, 1980), urinary, and liver function parameters (Brouwer *et al.*, 1991; Osterloh and Feldman, 1993). Only minor changes in urinary and liver function parameters were found in these studies (Brouwer *et al.*, 1991; Osterloh and Feldman, 1993) which were strongly criticized (Stott, Waechter, and Quast, 1990; van Sittert *et al.*, 1991).

Phosphine

A number of cases of acute phosphine poisoning are reported in the literature, most of them being suicides or suicide attempts (IPCS, 1988). The most common autopsy finding was congestion of the lungs with marked oedema. Symptoms of acute phosphine poisoning include symptoms relating to the nervous system (headache, vertigo, tremors, and unsteady gait, progressing in severe cases to convulsions, coma, and death), gastro-intestinal symptoms (loss of appetite, thirst, nausea and vomiting, diarrhoea, and severe epigastric pain), and respiratory symptoms (dyspnoea) (IPCS, 1988). Few acute poisonings after occupational exposure to phosphine used as a fumigant have been reported (Ames, 1991; Wilson *et al.*, 1980). Occupational exposure to phosphine was sometimes associated with mild aspecific symptoms (headache, dizziness, cough) without electrophysiological abnormalities (Misra *et al.*, 1988). In other cases mild alterations of some clinical chemistry parameters of uncertain significance were reported (IPCS, 1988).

Chlorophenoxy compounds

Since 2,4-D is the most widely used compound, toxicological data in humans mainly relate to exposure to this compound. Fewer data are available for MCPA.

Except for skin irritation and eczema (see Table 11.1), no other adverse effects have been observed in exposed workers during normal use. Few accidental high

exposures were characterized by aspecific signs and symptoms (vomiting, headache, dizziness, loss of consciousness) (Stevens and Sumner, 1991).

Chronic exposure to phenoxy acids has sometimes been associated with increased risk of non-Hodgkin's lymphoma, Hodgkin's disease, and soft-tissue sarcoma. However, several different reviews of available data have been published and concluded that there was no evidence of such an association (FAO/WHO, 1997; IARC, 1986; Munro *et al.*, 1992; Ritter, 1997). Since then a few more studies have been published, also showing inconsistent results (Becher *et al.*, 1996; Burns, Beard, and Cartmill, 2001; Fleming *et al.*, 1999; Zahm, 1997).

Available data indicate that 2,4-D is rapidly absorbed both by inhalation and dermal exposure (Frank, Campbell, and Sirons, 1985; Kolmodin-Hedman and Erne, 1980) although no precise estimate of the absorption rate is available. Based on a review of available data on human exposure, Munro *et al.* (1992) concluded that forestry workers had the highest exposure (estimated internal dose up to 40 μg/kg bw per day) whereas farmer and commercial applicator exposure was up to 6.3 μg/kg bw per day. Phenoxy acids are mainly excreted unchanged in the urine and levels up to 32 mg/L were found in the urine of exposed workers without signs or symptoms of toxicity (IARC, 1986). The Finnish Institute of Occupational Health proposed a post-shift, end-of-week biological limit value of 14 μmol/L of urinary phenoxy acids and the Scientific Committee on Pesticides of the International Commission of Occupational Health proposed a limit of 0.5 mg of 2,4-D or MCPA/L of urine (Lauwerys and Hoet, 2001).

Phenolic pesticides

Dinitrophenols

These compounds exert their toxic effects by uncoupling oxidative phosphorylation (Hollingworth, 2001). Their acute toxicity is relatively high and consequently a significant number of poisonings, mainly due to 4,6-dinitro-*o*-cresol (DNOC), occurred in the past leading to a decline in the use of these compounds. Signs of poisoning include fatigue, restlessness, and excessive sweating. Fatal hyperthermia may occur (Gasiewicz, 1991). Occupational exposures with blood levels <0.8 μg of DNOC/g were found not to be associated with signs and symptoms. Available data indicate that adverse effects are not seen at DNOC blood concentrations up to 20 μg/g after acute exposure (IPCS, 2000). However, in one instance a professional applicator exposed for about 3 weeks, complained of malaise, shortness of breath, and body weight loss, and blood DNOC levels were found to be 0.8 μg/g (IPCS, 2000). Lauwerys and Hoet (2001) proposed a biological exposure limit of 1 mg/100 ml of serum or 0.5 mg/100 ml of whole blood.

Other phenolic pesticides

The most important compounds in this group are bromoxynil and ioxynil. There are a number of reports of human poisoning by these compounds, usually in combination.

Most cases were suicidal, but few accidental occupational poisonings have been described. Signs and symptoms were those typical of poisoning with uncouplers of oxidative phosphorylation (Hollingworth, 2001).

Amitrole

Amitrole causes thyroid dysfunction in experimental animals. However, thyroid function was investigated in workers exposed to amitrole and was found to be within normal limits (IPCS, 1994b).

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12 Treatment of Pesticide Poisoning

Gregory P. Wedin and **Blaine E. Benson**

Introduction

Pesticide poisoning presents many treatment challenges. The lethal intent of these compounds, coupled with their widespread use and ready accessibility, results in many human exposures, both unintentional and deliberate self-poisonings. The human toxicity of these compounds varies widely, depending on the mechanism of action and its relevance to the biological systems in the human species.

This chapter provides guidance in the treatment of pesticide-poisoned patients. A detailed review is presented for the treatment of common pesticide exposures, including the bipyridyl herbicides, anticholinesterase insecticides, and anticoagulant rodenticides. Special considerations for the treatment of patients exposed to other types of pesticides are also presented.

General treatment guidelines

The treatment of poisoned patients consists of immediate life support measures, decontamination procedures, treatment of specific toxic effects or complications, procedures to enhance elimination of toxin, monitoring the patient for signs or symptoms of toxicity and response to therapy, and in some cases administration of specific antidotes. The severity of toxicity, as well as treatment decisions, will depend on a variety of factors surrounding the poisoning incident, including those listed in Table 12.1.

Life support measures

The initial steps in treating any poisoned patient include supportive measures to stabilize the patient and maintain vital functions, including airway, breathing, and

Table 12.1 Factors to be considered when treating poisoned patients

◆ Route of exposure
◆ Dosage form, e.g. solid, liquid, vapour, gas
◆ Time of/since exposure
◆ Total dose (amount × concentration)
◆ Presenting signs and symptoms
◆ Reason for exposure
◆ Age
◆ Underlying medical conditions

circulation. Seizures, which may result from strychnine, organochlorine insecticides, and other pesticides require prompt and aggressive treatment to stabilize the patient.

Respiratory support may include intubating the patient and providing oxygen and artificial ventilation. In cases of anticholinesterase insecticide poisoning it may also include administering atropine to control bronchial secretions. Hypotension and arrhythmias that are life threatening or compromise circulation should be treated promptly. In cases of anticoagulant rodenticide poisoning, blood products may need to be administered to control bleeding and to correct other haemodynamic disturbances.

Decontamination

Once stabilized, steps to decontaminate the patient and limit further exposure to the pesticide should commence. Gastrointestinal decontamination is important for recent acute ingestions, but will provide little benefit if done more than 1 h after the exposure or in poisonings resulting from repeated exposure to small amounts of pesticide.

Activated charcoal and other adsorbents, such as bentonite clay or cholestyramine, are important for initial gastrointestinal decontamination following ingestion of pesticides. Multiple doses of activated charcoal have been shown to enhance the elimination of long-acting anticoagulants and strychnine, but the clinical utility of this practice is questionable. Arsenic, barium, and other heavy metals do not bind to adsorbents. Whole-bowel irrigation to reduce systemic absorption may be beneficial following recent ingestion of a toxic dose of arsenic, but the potential benefit is only theoretical (Tenenbein, 1997).

Gastric lavage is generally reserved for use within 60 min of ingestion of a life-threatening dose of poison (Vale, 1997). Comatose patients and those with an impaired gag reflex should be intubated before the procedure to protect the airway. Gastric lavage is contraindicated if the pesticide is in a hydrocarbon-based solvent because of the increased risk of aspiration with this procedure.

Ipecac syrup has very limited usefulness for most pesticide exposures, although it may be useful for treatment of children in the home after recent ingestions,

provided that the child is alert and not expected to deteriorate quickly for any reason (Krenzelok, McGuigan, and Lheur, 1997). Ipecac is contraindicated if the patient has a depressed level of consciousness, after exposure to substances that cause seizures, such as strychnine, or when a pesticide is in a hydrocarbon-based solvent because of the increased risk of aspiration.

Irrigation and washing with soap and water generally is adequate to decontaminate the skin after dermal exposures to most pesticides. Anticholinesterase insecticides are highly lipophilic and are readily absorbed through the skin. Since the skin is not much of a barrier to absorption for these substances, decontamination should be performed as soon as possible following exposure. Dusts, powders, and other solids should be brushed or dusted off the skin before irrigation with water owing to the reactive nature of some substances, such as phosphorus. Contaminated eyes should be irrigated with water or normal saline for at least 15 min.

History and physical examination

Strive to obtain a complete and accurate history that includes the information listed in Table 12.1. Active ingredients in commercially available pesticides can vary substantially despite only subtle differences in product names. Whenever possible, it is always best to use the original package to identify the pesticide. Consult a local poison control centre for help in identifying the possible toxin(s) involved. The poison centre can also provide valuable assistance and information for the initial management of the poisoning as well as ongoing monitoring and treatment.

Standard toxicology screens using blood or urine generally do not include tests for pesticides. An initial set of laboratory data including serum electrolytes, serum creatinine, liver enzymes, prothrombin time, or international normalized ratio (INR) may assist in the initial evaluation and provide information about end organ effects of the pesticide. Cardiac monitoring in addition to routine vital signs may also be appropriate depending on the type of pesticide possibly involved and the clinical status of the patient.

The physical examination may provide clues to the cause of poisoning. The characteristic toxidrome associated with the anticholinesterase insecticides (coma, pinpoint pupils, increased secretions, incontinence, bradycardia, and muscle fasciculations), for example, may lead to a preliminary diagnosis. The smell of rotten fish may suggest exposure to a rodenticide containing phosphides, whereas the smell of garlic may suggest arsenic or phosphorous poisoning. Electrolyte abnormalities such as hypokalaemia or hypercalcaemia may suggest exposure to barium or cholecalciferol, respectively.

Treatment of specific complications

The primary approach to treating most pesticide poisonings consists of symptomatic and supportive care using standard therapeutic measures. Refer to the discussion of

Table 12.2 Antidotes for pesticide poisoning

Antidote	Pesticide
Atropine	Anticholinesterase insecticides (organophosphates and carbamates)
Glycopyrrolate	Anticholinesterase insecticides (organophosphates and carbamates)
Pralidoxime	Anticholinesterase insecticides (organophosphates)
Obidoxime	Anticholinesterase insecticides (organophosphates)
Dimercaprol	Arsenic
Succimer	Arsenic
Vitamin K_1	Anticoagulants
Potassium	Barium
Niacinamide	PNU
Digoxin FAB fragments	Red squill
Methylene blue	Urea-substituted herbicides, substituted anilines

toxicity for specific pesticides in other chapters of this volume for information on the potential toxic effects of individual agents. Special considerations or therapeutic strategies for individual pesticides will be presented in the following sections.

Enhancing elimination

Special techniques such as repeated doses of activated charcoal, forced diuresis, haemodialysis, and haemoperfusion can be used to enhance the elimination of some pesticides. Specific information on the indications and techniques for such procedures are provided in the discussion for treatment of individual pesticides throughout this chapter.

Antidotes

Few antidotes exist for treating pesticide poisonings. Table 12.2 lists commonly used antidotes for pesticide poisonings. The following sections include a detailed discussion of pertinent antidotes where applicable.

Insecticides

Organophosphates and carbamates

There are no established toxic or lethal doses for organophosphates or carbamates. Taste amounts or unintentional sprays of household strength products do not produce significant clinical effects and these patients can be observed at home.

Deliberate ingestions or unintentional exposures to agricultural grade products, however, require close observation and treatment in a hospital setting. Mortality associated with organophosphate and carbamate poisoning ranges from 4 to 30 per cent (Yamashita *et al*., 1997). Death results from respiratory compromise, seizures, and cardiac arrhythmias.

There are no readily available blood or urine tests for quantifying the absorbed dose of organophosphate or carbamate compounds. Plasma or erythrocyte cholinesterase activity can serve as a rough estimate of the severity of exposure and to monitor the patient's progress, but usually patients are treated based on symptoms. Daily cholinesterase activities should be considered for monitoring severe exposures where prolonged antidote therapy is required. Erythrocyte cholinesterase activities are more difficult to perform than plasma cholinesterase, but are thought to more accurately reflect motor end-plate activities. In severe exposures, acetylcholinesterase activities drop to <10 per cent of patients' baseline. Clinical improvement has been associated with cholinesterase activities rising to 10–30 per cent of patients' baseline (Cunha *et al*., 1995; Du Toit *et al*., 1981).

Two other tests that may be of prognostic value are serum amylase and an electrocardiogram (ECG). Hyperamylasaemia has been associated with the development of shock (Lee *et al*., 1998) and respiratory failure (Matsumiya *et al*., 1996) in organophosphate poisoning. QTc prolongation often heralds respiratory failure (Chuang *et al*., 1996). A promising new prognostic technique is spectral analysis of systematic arterial pressure and heart rate. Instantaneous blood pressures and heart rates are converted to two-dimensional spectrograms. Low frequency components of blood pressure correlate with neurogenic vasomotor tone and functional integrity of the brain stem. The early low frequency and very low frequency components of blood pressure and heart rate spectra predicted mortality and long-term vegetative states early in the course of 20 respiratory dependent organophosphate poisoned patients (Yen *et al*., 2000).

Intubation, suctioning, and oxygen administration are important elements of supportive care. Seizures should be treated with a benzodiazepine. Organophosphate poisoned patients with signs of systemic toxicity should be observed for at least 24 h after recovery to monitor for the development of the intermediate syndrome.

Initial decontamination includes removing the patient's contaminated clothing while wearing rubber gloves and a rubber apron to prevent secondary contamination. Contaminated clothing should be double-plastic-bagged and properly disposed. Exposed skin should be washed several times with soap and water. Activated charcoal should be administered for recent ingestions. Careful gastric lavage with a small-bore nasogastric tube may be useful following ingestion of liquid products. The benefits of gastric lavage, however, must be carefully weighed against the potential risk of aspiration, especially if the product is dissolved in a hydrocarbon base. Laboratory tests to consider in symptomatic patients include oxygen saturation, arterial blood gases, electrolytes, a chest X-ray, cholinesterase

activity, serum amylase, serum lipase, ECG, and an ultrasound of the abdomen (pancreatic status).

Haemodialysis, haemoperfusion, forced diuresis, and multiple doses of activated charcoal are unlikely to enhance the elimination of these compounds because of their large volumes of distribution. In one series involving 10 patients, haemoperfusion removed only 1–8 mg of organophosphate and did not lead to clinical improvement (Martinez-Chuecos *et al.*, 1992).

Symptomatic patients may benefit from antidote therapy. There are two classes of antidotes used: the antimuscarinics and oximes. Antimuscarinics block muscarine receptors in the central and peripheral nervous systems. Adequate dosing will alleviate sweating, salivation, bronchorrhea, and bradycardia. Atropine is a tertiary amine and acts both centrally and peripherally. It is administered in 1–2 mg doses in adults and 0.02–0.05 mg/kg in children every 5–10 min until tracheobronchial secretions dissipate. Atropine infusions (0.02–0.08 $mg\,kg^{-1}\,h^{-1}$) have been used in severely poisoned patients (Chew, Chee, and Yeo, 1971; Du Toit *et al.*, 1981). A total of 30 g of atropine was infused over 5 weeks in one case (LeBlanc, Benson, and Gilg, 1986). Preservative-free atropine should be used when prolonged infusions are needed.

Glycopyrrolate is a quaternary amine antimuscaric, which does not cross the blood–brain barrier. Theoretically, it will dry secretions and cause less confusion and tachycardia than atropine. Doses of 0.05 mg/kg have been used (Choi *et al.*, 1998; Tracey and Gallagher, 1990). A double-blind randomized trial comparing atropine with glycopyrrolate in the treatment of organophosphate poisonings did not reveal a difference in outcomes or complications between the two therapies. There were only 39 patients in the trial, however, so low study power may have prevented the investigators from detecting a statistically significant difference (Bardin and van Eeden, 1990).

Oxime therapy is more controversial. The traditional view has been that oximes such as pralidoxime chloride (2-PAM), pralidoxime mesylate (P2S), and obidoxime (Toxigonin) combine with the organophosphate or carbamate at the active site of acetylcholinesterase, effect a conformational change, and allow the organophosphate or carbamate to leave, thereby regenerating the enzyme. More recently it has been proposed that oximes may also have additional non-regenerating effects including atropine-like activity, ganglionic receptor blocking action, and direct organophosphate detoxifying capability (van Helden *et al.*, 1991).

Oximes do not consistently provide therapeutic benefit. Animals poisoned with carbamate and then treated solely with an oxime had worse outcomes than controls treated with normal saline (Natoff and Reiff, 1973; Sanderson, 1961; Sterri *et al.*, 1979). Oximes failed to reactivate human erythrocyte acetylcholinesterase inhibited with aldicarb, methoxyl, carbaryl, malathion, and dicrotophos (Ganendran and Balabaskaran 1974a, 1974b; Lifshitz *et al.*, 1994). Also, oximes have failed to improve clinical outcomes in humans poisoned by organophosphates in recent clinical trials (Cherian *et al.*, 1997; de Silvia, Wijewickrema, and Senanayake, 1992).

One possible reason for this inconsistency may be that current dosing regimens are too simplistic. Several groups have shown that oxime effectiveness in humans is

dependent on maintaining a proper ratio of organophosphate to oxime (Thiermann *et al.*, 1997; Willems *et al.*, 1993). It is likely that oximes must be dosed both in sufficient quantity and duration to prevent re-inhibition of acetylcholinesterase from sequestered organophosphate.

Until a scientifically valid clinical trial is conducted comparing atropine therapy with atropine plus optimally dosed oxime therapy, the role of oximes will likely remain controversial. Since oximes usually provide synergistic benefit when used with atropine, it is reasonable to start oxime therapy in patients who require atropine and continue it for the duration of atropine therapy. Pralidoxime (Cl, I, mesylate, methylsulphate) is administered slowly by intravenous infusion in doses of 1–2 g, repeated as needed every 3–8 h to a maximum dose of 12 g/day. Obidoxime is administered intravenously in a dose of 250 mg, repeated once or twice at 2-h intervals up to a maximum daily dose of 750 mg.

Continuous infusion of oxime provides more uniform blood concentrations and may be especially important in severely intoxicated patients where continued insecticide absorption and breakthrough enzyme inhibition may be taking place. One regimen that has been shown to be pharmacokinetically sound for pralidoxime chloride in healthy adults is to administer 4 mg/kg intravenously (IV) over 15 min followed by a maintenance infusion of 3.2 $mg\,kg^{-1}\,h^{-1}$ (Medicis *et al.*, 1996). In children the regimen is 25–50 mg/kg followed by continuous infusion of 10–20 $mg\,kg^{-1}\,h^{-1}$ (Schexnayder *et al.*, 1998). The infusion regimen derived for pralidoxime methylsulphate is a loading dose of 4.42 mg/kg followed by a maintenance infusion of 2.14 $mg\,kg^{-1}\,h^{-1}$ (Willems *et al.*, 1992). For obidoxime, a 250 mg load followed by 740 mg/24 h has been shown to keep oxime concentrations within the therapeutic range of 10–20 μmol (Thiermann *et al.*, 1997).

Diazepam and magnesium may also play important roles in the care of certain organophosphate and carbamate poisoned patients. Magnesium (2–4 g IV) reversed premature ventricular contractions and pleomorphic ventricular tachycardia ('torsades de pointes') in a 63-year-old patient poisoned by parathion (Wang, Tseng, and Bair, 1998). In pigs, magnesium was also useful in stopping organophosphate-induced tachycardia (Petroianu and Ruefer, 1992) and in normalizing blood pressure (Petroianu *et al.*, 1998). In monkeys, diazepam has been shown to prevent soman-induced neurological damage when used in conjunction with pralidoxime and atropine (Hayward *et al.*, 1990; Murphy *et al.*, 1993).

There are a number of potentially useful treatments in development. Hagedorn oximes HI 6 and HLö 7 have more potent reactivation capability than pralidoxime and obidoxime (Worek *et al.*, 1998). Human butyrylcholinesterases are being tested as potential scavengers for organophosphates (Allon *et al.*, 1998). Clonidine, fluoride, and anneal red cells containing phosphotriesterase all show promise as future adjunct therapies (Karalliedde, 1999). Partial α_1-receptor agonists could be useful in preventing organophosphate- and carbamate-induced seizures and nerve damage (Lallement *et al.*, 1999).

Pyrethroids

Pyrethroids are of low toxicity compared with other classes of insecticides. They are poorly absorbed across the skin, and have an oral bioavailability of only 36 per cent (Ray and Forshaw, 2000). The small fraction of dose that is absorbed is rapidly detoxified by carboxyesterases in the liver. Although pyrethroids can cause life-threatening hypersensitivity reactions with normal use, most unintentional dermal exposures will result in no harm to the patient who can be observed at home. Deliberate self-poisoning, however, warrants treatment and monitoring in hospital owing to the potential for muscle fasciculations, coma, pulmonary oedema, and the delayed onset of seizures (He *et al.*, 1989; Poulos, Athanaselis, and Coutselinis, 1982).

Hypersensitivity reactions may occur immediately after exposure and can be life-threatening (Culver, Malina, and Talbert, 1988; Wax and Hoffman, 1994). Individuals with a prior history of asthma or ragweed allergies may be particularly sensitive to pyrethroids. Mild dermatitis responds well to antihistamines. Systemic hypersensitivity reactions (respiratory distress, shock) require epinephrine and corticosteroids.

Activated charcoal should be administered to patients who deliberately ingest pyrethroids. The remainder of treatment consists primarily of symptomatic and supportive care (Bateman, 2000). Benzodiazepines are indicated for immediate treatment of seizures. Phenobarbital may provide even better protection because of its direct agonist action on chloride channels (Ray and Forshaw, 2000). There have been no studies examining the efficacy of standard methods for enhancing the elimination of pyrethroids.

Dermal exposure to type II agents, such as cyhalothrin, cypermethrin, deltamethrin, and fenvalerate, may result in burning, tingling, itching, or numbness of the affected area 1–2 h after the exposure. These manifestations may last up to 24 h and can be treated with topical vitamin E in a vegetable oil base (Wilks, 2000).

Organochlorines

Organochlorine insecticides are highly lipophilic, well absorbed across intact skin, and capable of inducing hepatic microsomal enzymes. During a poisoning, organochlorines cause seizures, respiratory failure, and death. The onset of symptoms is dependent on the route of exposure. Ingestion of 5–10 g of lindane produced vomiting and seizures within 15 min of ingestion (Jaeger *et al.*, 1984). Seizures have been delayed as long as 5–6 h after ingestion (Smith, 1991). Total body dermal applications of lindane produced seizures and death 24–48 h after application (Davies *et al.*, 1983; Telch and Jarvis, 1982).

The toxic and lethal doses for organochlorine insecticides are on the order of 2–10 g for adults. Seizures have occurred with as little as 450 mg of lindane in a 4-year-old and 600 mg of lindane in an adult (Kurt *et al.*, 1986). Organochlorine

blood tests are generally not available and correlate poorly with toxicity. The laboratory work-up of a symptomatic patient should include a baseline complete blood count (CBC), ECG, electrolytes, serum creatinine, blood urea nitrogen (BUN), and liver transaminases.

In addition to standard supportive measures, initial treatment may need to be directed at controlling seizures with benzodiazepines and/or phenobarbital. All contaminated clothing should be removed using proper protective measures (rubber gloves, rubber apron). Decontaminate the skin thoroughly by washing several times with soap and water. Gastric lavage should be considered for recent liquid ingestions. Ipecac syrup should not be used owing to the risk of seizures during emesis.

It is unclear whether activated charcoal binds organochlorines. Charcoal has been shown to reduce absorption of a variety of doses of dieldrin given to sheep, goats, and heifers (Cooney, 1995). In a mouse model comparing cholestyramine with activated charcoal, however, cholestyramine was more effective in preventing lindane absorption and in preventing seizures than activated charcoal (Kassner *et al.*, 1993). Repeat dose cholestyramine was used to enhance elimination of chlordecone, an organochlorine with enterohepatic recirculation (Guzelian, 1981). There is no evidence supporting the use of multiple dose activated charcoal to enhance the elimination of organochlorines. Haemoperfusion has not enhanced clearance of endosulfan or chlordecone (Boereboom *et al.*, 1998; Skalsky *et al.*, 1979).

Boric acid and nicotine

Boric acid will produce vomiting, green diarrhoea, dehydration, shock, seizures, renal failure, and death. Patients may develop a boiled lobster appearing rash on their palms, soles, and trunk within 2 days of exposure. The acute lethal dose of boric acid is 5 g in children and 30 g in adults. Treatment consists primarily of symptomatic and supportive care. Activated charcoal does not appreciably bind boric acid. Haemodialysis may be useful in enhancing the elimination of the compound in severe cases (Siegel and Wason, 1986).

Nicotine produces nausea, vomiting, salivation, diarrhoea, muscle weakness, and confusion. Patients may quickly succumb to respiratory paralysis. Treatment consists primarily of symptomatic and supportive care. Thorough skin decontamination should be performed for dermal exposure. Activated charcoal is helpful for ingestions (Saxena and Scheman, 1985).

Herbicides

Chlorophenoxy herbicides

Deliberate self-poisoning with the chlorophenoxy herbicides requires prompt and intensive therapy to help prevent or manage potentially life-threatening toxic

effects including coma, convulsions, pulmonary oedema, hypotension, and ECG changes. Unintentional or casual exposures to small quantities of these substances generally pose little risk of serious toxicity and often require only observation.

Although patients typically vomit after the ingestion of large, toxic doses of these substances, steps to decontaminate the gastrointestinal tract should still be taken if the patient presents for treatment within approximately 1 h of exposure. Activated charcoal should be administered if a potentially toxic dose has been ingested. Gastric lavage should be considered in patients exhibiting serious signs or symptoms of toxicity or if a life-threatening dose is ingested. Since many of these substances are often found in hydrocarbon solutions, protecting the airway should be a priority.

Initial evaluation and ongoing monitoring should include an ECG, serum electrolytes, including calcium, serum creatinine, liver enzymes, CBC, urine albumin and myoglobin, serum creatinine phosphokinase, and arterial blood gases in symptomatic patients. Serum concentrations of the chlorophenoxy compounds may help confirm exposure or indicate the potential severity of poisoning, but are not readily available. Treatment should be based on the clinical manifestations of poisoning.

Treatment consists primarily of symptomatic and supportive care. In severe overdose, chlorophenoxy herbicides demonstrate dose-dependent elimination kinetics (Flanagan *et al.*, 1990). Alkaline diuresis has been shown to significantly increase renal clearance of chlorophenoxy compounds (Flanagan *et al.*, 1990; Prescott, Park, and Darrien, 1979).

Case reports have demonstrated a striking parallel between increased renal clearance of these compounds with alkaline diuresis and clinical improvement in patients (Prescott, Park, and Darrien, 1979; Schmoldt, Iwersen, and Schluter, 1997). Systemic alkalization and the shift of these compounds from the tissues into the circulation may also contribute to the improvement in these patients (Flanagan *et al.*, 1990). In addition, urine alkalinization may help guard against nephrotoxicity associated with rhabdomyolysis.

Haemodialysis has also been used successfully to treat patients who ingested potentially lethal doses of 2,4-D (Durakovic *et al.*, 1992). This may provide an alternative approach for enhancing elimination of these compounds in patients who cannot tolerate alkaline diuresis.

Paraquat

Nearly all paraquat poisonings result from ingestion. Dermal exposure may result in dermatitis but rarely results in systemic toxicity unless the skin is abraded and there is prolonged exposure to concentrated product (Garnier, Chataigner, and Efthymiou, 1994). Ocular exposure may result in a delayed sloughing of corneal epithelium 12–24 h after exposure, but also does not result in systemic toxicity. Inhalation of paraquat droplets may produce nasal and tracheobronchial irritation

but does not produce systemic toxicity because of low product vapour pressure, large droplet size, and low application concentration.

Paraquat produces three dose-dependent syndromes (Pond, 1990; Vale, Meredith, and Buckley, 1987). Patients ingesting <20 mg/kg paraquat ion typically develop self-limiting gastrointestinal complaints (nausea, GI irritation, diarrhoea) and recover without sequelae. Patients ingesting 20–40 mg/kg paraquat ion develop upper GI irritation/corrosion, acute tubular necrosis (12–48 h after ingestion), pulmonary haemorrhage (24–48 h after ingestion), and pulmonary fibrosis (1–2 weeks after exposure). Most of these patients die within 5–14 days. Patients ingesting >40 mg/kg die within several hours to several days from multiple system organ failure.

Several laboratory tests can be used to gauge the severity of paraquat exposure. A simple bedside test can be performed by adding 250 μl of a freshly prepared sodium dithionite solution (100 mg sodium dithionite into 5 ml of 5 M NaOH) to 1 ml of the patient's urine. If the urine turns a blue-black colour, paraquat is present in concentrations of 2 μg/ml or greater (Pond, 1998). The darker the colour, the worse is the patient's prognosis (Yamashita, 1989). The patient's urine should be tested serially for 24 h after ingestion. Positive urine tests should be followed with quantitative plasma paraquat levels. Paraquat levels usually are only available through the product manufacturer. Their telephone number is often listed on the product container or can be obtained from a local poison centre.

Plasma paraquat levels greater than 3 μg/ml are inevitably associated with death. The probability of survival can be estimated by a number of statistical methods (Jones, Elton, and Flanagan, 1999; Yamamoto *et al.*, 2000). The easiest is to plot the patient's plasma paraquat concentration on the survival curve shown in Figure 12.1 (Hart, Nevitt, and Whitehead, 1984).

Unless the exposure is trivial, all paraquat ingestions require immediate treatment and close monitoring in a hospital setting. Initial treatment consists of removing contaminated clothing and performing standard decontamination procedures for skin and eyes. Eye exposures require close follow-up for at least 24 h owing to the potential for delayed corneal sloughing. Vomiting and oral burns are frequent with ingestions of concentrated paraquat solutions (20 per cent). Since paraquat avidly binds to clay, oral administration of dirt may be useful as a pre-hospital treatment for minimizing paraquat absorption until activated charcoal can be administered. Activated charcoal has been shown to bind paraquat better than fullers' earth or bentonite (Okonek *et al.*, 1982). There are no data showing that charcoal improves patient outcome, however. Lavage should not be performed because of the risk of further injuring a corroded oesophagus or stomach (Meredith and Vale, 1987).

Intravenous fluids and electrolytes will be required to maintain blood pressure and kidney perfusion. Analgesics should be administered to relieve oral pain. A central line and a Swan – Ganz catheter should be placed since the rapid development of cardiac and renal failure may otherwise make it difficult to optimally titrate fluids, pressors, inotropes, and vasodilators. Supplemental oxygen may

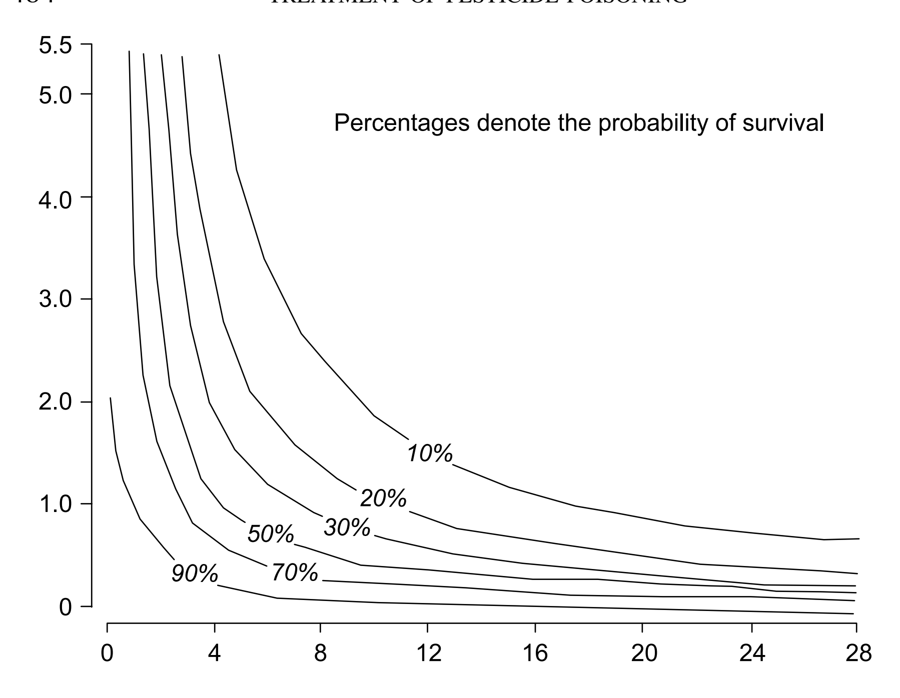


Figure 12.1 Contour graph showing relation between plasma paraquat concentration (μg/ml), time after ingestion, and probability of survival. (Legend and figure reproduced with permission from Lancet. Hart, T. B., Nevitt, A., and Whitehead, A. (1984). A new statistical approach to the prognostic significance of plasma paraquat concentrations. *Lancet*, **2**, 1222–1223)

enhance the generation of superoxide radicals and should be avoided unless the patient's oxygen tension falls below 50 mmHg.

The only method that has been shown to enhance the elimination of paraquat is charcoal haemoperfusion (Hampson, Effeney, and Pond, 1990; Hampson and Pond, 1988; Widdop, Medd, and Braithwaite, 1976). A 6–8-h course of haemoperfusion may be beneficial if the procedure can be instituted within 4 h of ingestion.

Pulse therapy with cyclophosphamide and methylprednisolone shows promise in reducing paraquat mortality. This therapy is thought to work by reducing the inflammatory process leading to pulmonary fibrosis. In a single blinded randomized clinical trial involving 142 paraquat-poisoned patients, pulse therapy reduced mortality in moderate to severe paraquat poisoning from 57 per cent to 18 per cent (Lin *et al.*, 1999). All patients received activated charcoal, two 8-h courses of charcoal haemoperfusion upon presentation, and 10 mg of dexamethasone every 8 h for 14

days. Pulse therapy patients also received $15\,g\,kg^{-1}\,day^{-1}$ of cyclophosphamide given for 2 days and 1 g/day of methylprednisolone for 3 days. The study was criticized for possible selection bias during data analysis (Buckley, 2001).

Diquat

Diquat is another bipyridal herbicide having similar kinetic, pharmacological, and physical properties to paraquat. The biggest difference between the two compounds is that diquat is not actively taken up by the lung so it does not produce pulmonary toxicity. Also, when diquat reacts with sodium dithionite it produces a yellow-green colour instead of dark blue. There is not enough experience with diquat to relate diquat plasma concentrations with prognosis. Instead, diquat poisonings are categorized by symptoms and dose ingested. Patients ingesting <1 g diquat ion usually develop only gastrointestinal signs and symptoms and transient renal impairment. Patients ingesting 1–12 g may develop coma, renal failure, seizures, and ventricular arrythmias, but usually recover. Patients ingesting a dose of >12 g diquat ion usually develop multi-system organ failure resulting in death. Treatment is similar to paraquat except there is no need for pulse dose cyclophosphamide and methylprednisolone, and less concern about using supplemental oxygen (Jones and Vale, 2000).

Glyphosate and glufosinate

The organophosphorous herbicides glyphosate and glufosinate are generally considered to be minimally toxic to mammals. The LD_{50} reported from animal studies on these agents is in the range 4000–6000 mg/kg for glyphosate and 436–1660 mg/kg for glufosinate (Koyama *et al.*, 1994; Talbot *et al.*, 1991). Standard decontamination procedures should be carried out in cases of skin or eye exposoure. Skin or eye irritation should be treated symptomatically.

The unintentional ingestion of a small amount of glyphosate- or glufosinate-containing herbicides is unlikely to produce clinically significant toxicity. In such cases, water should be administered to dilute the product and minimize irritation.

Deliberate self-poisoning with large amounts of either glyphosate or glufosinate, however, should be treated aggressively. Massive overdose with commercial products that contain these herbicides have resulted in vomiting, erosion of the gastrointestinal tract, laryngeal injury, aspiration pneumonitis, pulmonary oedema, hypotension, coma, and death (Talbot *et al.*, 1991). Other constituents in these products, however, are strongly suspected to contribute to the toxicity or even be the primary causative factor (Hung, Deng, and Wu, 1997; Koyama *et al.*, 1994).

Patients who ingest large amounts of glyphosate are at risk for aspiration pneumonia due to repeated vomiting and laryngeal injury. If repeated vomiting does not occur, gastric lavage should be considered. Steps should be taken to protect the airway if gastric lavage is performed, or in cases where laryngeal injury is

suspected. Activated charcoal can be administered, although its effectiveness is not well documented. Supportive measures should be directed at maintaining fluid and electrolyte balance as well as adequate respiratory and cardiovascular function.

Following deliberate ingestion of a potentially toxic dose of glufosinate, gastric lavage should be performed if it can be done within an hour of ingestion. Activated charcoal should be administered. The early use of haemodialysis and haemoperfusion has been shown to effectively remove glufosinate from the blood, but delayed central nervous system depression, respiratory arrest, and seizures have subsequently developed regardless. Treatment consists primarily of symptomatic and supportive care. Diazepam is effective at controlling seizures, but recurrent seizures suggest the need for maintenance doses of longer acting anticonvulsants such as phenobarbital or phenytoin.

Other herbicides

Several types of herbicides, including sodium chlorate, urea-substituted herbicides, and the substituted aniline herbicides are all capable of producing methemoglobinaemia (Marrs and Dewhurst, 2000). Treatment should consist of decontamination, supportive care, and the administration of methylene blue in cases of significant methemoglobinaemia. Alkaline diuresis should also be considered in cases that are complicated by intravascular hemolysis.

Rodenticides

Anticoagulant rodenticides

Ingestion is the most common route of toxic human exposure to the anticoagulant rodenticides. The initial triage and the extent of subsequent care and monitoring are dependent on whether the ingested product contains warfarin or a long-acting anticoagulant such as brodifacoum, diphacinone, or chlorphacinone. The general approach to treatment of the resultant coagulation disorder is virtually the same regardless of the offending agent, except for the magnitude and duration of the use of blood products and the antidote vitamin K_1 (phytonadione). The initial triage of patients, however, remains controversial.

The acute unintentional ingestion of a warfarin-containing rodenticide is highly unlikely to cause a significant anticoagulant effect. The typical concentration of warfarin in these rodent bait products is 0.025 per cent. The dose predicted to prolong the prothrombin time and be potentially toxic in children is approximately 0.5 mg/kg (Carpentieri, Ngheim, and Harris, 1976). For a 10 kg child, this would amount to approximately 20 g of bait. Since gastrointestinal absorption of warfarin from rodenticide products is predictably less than complete, much higher amounts of bait would actually need to be ingested to produce a significant anticoagulant effect.

Although the potential for toxicity with the long-acting anticoagulants is much higher, a single, acute, unintentional ingestion is also unlikely to produce an anticoagulant effect that would harm a small child. Long-acting anticoagulants are up to 100 times more toxic than warfarin on a molar basis, but the concentration of these compounds in commercially available rodenticides is only 0.005 per cent. The minimum dose required to depress prothrombin complex activity in rats was 0.1 mg/kg, which is equivalent to 20 g of bait in a 10 kg child. Once again, this assumes 100 per cent absorption, which is not to be expected.

Consistent with this analysis, there has yet to be a single reported incident of significant illness or death associated with a single, acute, unintentional ingestion of an anticoagulant rodenticide. In addition, poison centre analysis of such cases has revealed nothing more than benign clinical outcomes (Morrissey, Burgess, and Robertson, 1995; Shepherd, Klein-Schwartz, and Anderson, 1998; Ingels *et al.*, 2002).

In summary, a single, acute, unintentional ingestion of an anticoagulant rodenticide does not require treatment. Parents or guardians of children should be instructed to observe for clinical evidence of bleeding, such as epistaxis, easy bruising, or abnormal bleeding from the gums. If such abnormalities occur within a few days of ingestion, the patient should be evaluated with an analysis of the prothrombin time (PT).

Deliberate self-poisonings or ingestions of suspicious nature, on the other hand, require more rigorous evaluation and treatment. Long-acting rodenticides may accumulate in the body with repeated exposure to even small amounts. These types of exposure should also be evaluated in a healthcare facility.

Active bleeding should first be controlled with fresh frozen plasma (FFP), which replaces deficient clotting factors. Activated charcoal should be administered if any product had been recently ingested, or if the history of ingestion is unreliable.

The PT can be used for initial evaluation in patients with a history of anticoagulant rodenticide ingestion, although it may not become prolonged for 24–48 h after ingestion. If bleeding has occurred, a haemoglobin and haematocrit should be obtained to assess the severity of blood loss. In the presence of a coagulopathy with an uncertain history, the initial laboratory evaluation should include a PT, partial thromboplastin time (PTT), thrombin time, and fibrinogen concentration. A prolonged PT and PTT with a normal thrombin time and fibrinogen concentration is consistent with vitamin K deficiency or an anticoagulant effect.

An analysis of coagulation factors is another means to narrow in on the diagnosis. Anticoagulant rodenticides will depress the vitamin K dependent clotting factors, specifically, II, VII, IX, and X. Factor analysis may be useful to more closely follow the course of toxicity and response to therapy and may provide a more sensitive screening test because changes in factor levels proceeds changes in the PT (Hoffman, Smilkstein, and Goldfrank, 1988).

Serum concentrations of brodifacoum and diphacinone can be measured using high-performance liquid chromatography (McCarthy *et al.*, 1997; Murphy *et al.*,

1989). Although not routinely available in most hospital settings, these concentrations can be used to quantify the severity of exposure and to guide therapy with vitamin K_1 (Bruno *et al.*, 2000).

Once active bleeding is corrected using fresh frozen plasma, additional blood products may be required to correct other haematologic abnormalities resulting from blood loss. Vitamin K_1 therapy should be started promptly to correct the underlying vitamin K deficiency and restore production of vitamin K dependent clotting factors.

Vitamin K_1 can be administered intravenously, subcutaneously, intramuscularly, or orally. The proper dose and route of administration is dependent on the severity of poisoning, clinical response to therapy, and possibly other circumstances such as the dependability of the patient to comply with oral therapy.

The intravenous route offers rapid delivery of vitamin K_1 to the blood stream, but also poses the risk of an anaphylactoid reaction. For this reason, the intravenous route should generally be avoided. The subcutaneous route offers faster delivery of the vitamin to the blood stream than the oral route, and does not pose the same risks associated with intravenous administration. The subcutaneous route may not be practicable if large doses are required, necessitating large volumes of fluid to be injected. The oral route can be used effectively if the patient has a functioning gastrointestinal tract and is willing and able to swallow large numbers of tablets. The bioavailability of vitamin K_1, however, is highly variable with oral doses (Park *et al.*, 1984).

Since the normal physiological recycling of vitamin K_1 is blocked by the anticoagulant rodenticide, higher than normal doses of vitamin K_1 are required to maintain an adequate source for clotting factor production. High doses of vitamin K_1 can be used safely and may obviate the need for repeated administration of FFP. Failure to adequately treat the underlying vitamin K_1 deficiency can result in a complicated clinical course.

A reasonable initial oral dose of vitamin K_1 is 50–100 mg. If parenteral therapy is necessary, a starting dose of 25–50 mg is appropriate. If the intravenous route is used, the dose should be diluted and administered slowly to reduce the risk of severe adverse reactions. Regardless of the route of administration, the dose must be repeated up to three or four times daily to provide a continuous source of vitamin K_1 for coagulation factor synthesis.

Prolonged therapy, ranging from many weeks to months, is often required owing to the long-lasting effects of these compounds, which is a result of their high lipid solubility and hepatic recirculation. The patient must be closely monitored as the dose of vitamin K_1 is tapered. As the dose is decreased the PT should be measured daily for several days to monitor for evidence of recurrent anticoagulation. Alternatively, factor analysis may provide a more sensitive and early indicator of the need for further treatment (Hoffman, Smilkstein, and Goldfrank, 1988).

Repeated doses of activated charcoal and cholestyramine have been shown to enhance the elimination of the anticoagulant rodenticides (Donovan, Ballard, and

Murphy, 1990; Renowden *et al*., 1985). The clinical significance of these findings, however, has yet to be demonstrated. The use of phenobarbital to enhance the hepatic metabolism of the long-acting anticoagulants and decrease the anticoagulant effect has been proposed (Lipton and Klass, 1984). The risks associated with the use of phenobarbital, however, outweigh the potential benefit of this therapy.

Arsenic

Arsenic was once used commonly in rodenticides, insecticides, and herbicides. Although it has largely been replaced with substances less toxic to humans and other mammalian species, poisonings still occur and successful treatment depends on early recognition and aggressive management.

Acute poisonings typically result from ingestion. Gastric lavage should be considered in patients who present for treatment soon after exposure. Activated charcoal does not bind heavy metals and is not indicated except in cases of mixed pesticide exposure.

Arsenic is radiopaque and an abdominal radiograph may provide evidence of recent ingestion and prove useful in guiding gastrointestinal decontamination. A negative abdominal radiograph should not rule out exposure, however, due to the rapid absorption of some arsenic compounds. Whole bowel irrigation has been successfully employed to cleanse the gastrointestinal tract of arsenic-containing compounds and should be considered in cases where radiopaque densities are evident in the intestines (Lee *et al*., 1995).

A 'spot' urine arsenic level may be useful for initial evaluation, but timed urinary excretion of arsenic is more accurate and should be used to guide therapy (Graeme and Pollack, 1998). Excretion of greater than 100 μg of arsenic in 24 h is generally considered abnormal. Initial evaluation should include a complete blood count (CBC), urinalysis, electrolytes, serum creatinine, liver enzymes, and an ECG.

Haemodynamic monitoring along with the administration of appropriate fluids, electrolytes, and vasopressors is an essential part of supportive therapy to correct volume depletion that results from third-spacing of fluids. Diuresis will help to enhance excretion and urine alkalinization will protect the kidneys from red cell breakdown products. Haemodialysis will not help enhance the elimination of arsenic, but may be necessary as a supportive measure for subsequent renal function impairment (Levin-Scherz *et al*., 1987).

Chelation therapy with dimercaprol (BAL) or one of its water-soluble analogues, 2,3-dimercaptosuccinic acid (DMSA) or dimercaptopropanesulfonate (DMPS), is definitive therapy for arsenic poisoning. BAL is administered intramuscularly, whereas DMSA and DMPS are administered orally. BAL may offer an advantage in patients with severe gastroenteritis, who will not tolerate oral therapy. On the other hand, DMSA or DMPS may be more effective and better tolerated (Brayer, Callahan, and Wax, 1997; Graeme and Pollack, 1998).

Strychnine

Early recognition of strychnine poisoning, along with aggressive airway management and seizure control, are important in minimizing complications and improving chances for survival. Care must be taken to minimize external stimuli, which can precipitate seizures.

Benzodiazepines, such as diazepam or lorazepam, are the drugs of choice for the acute treatment of seizures. Barbiturates, such as phenobarbital, are also effective and provide longer lasting seizure control. If these agents are not effective, then the patient should be paralysed with a non-depolarizing neuromuscular blocking agent.

Once the patient is stabilized, activated charcoal should be administered. Gastric lavage should be performed only if a life-threatening dose of strychnine was recently ingested and if the airway has been secured. Inserting the orogastric tube may precipitate seizures. Pre-medication with diazepam should be considered prior to attempting to intubate a patient suspected of strychnine intoxication. Administering activated charcoal before and after lavage has been suggested in such cases. Ipecac syrup is contraindicated.

Repeat doses of activated charcoal has been suggested as a means to enhance elimination of strychnine due to its entero-hepatic recirculation, but the effectiveness is only theoretical. Other means to enhance elimination, such as dialysis, haemoperfusion, or forced diuresis, are not indicated.

Initial evaluation and ongoing monitoring should include an ECG, serum electrolytes, including calcium, serum creatinine, liver enzymes, urine myoglobin, serum creatinine phosphokinase, and arterial blood gases. Alkaline diuresis should be used to protect the kidneys from the toxic effects of myoglobin.

Other rodenticides

Early recognition of the causal agent and meticulous attention to symptomatic and supportive care is important to the successful management of other, less common, rodenticide poisonings. Often, specific treatments remain unproven or controversial because of limited clinical experience.

Thallium

Outside of the United States, Prussian blue is used as an alternative to activated charcoal to reduce gastrointestinal absorption of thallium. Potassium is exchanged with thallium, forming an insoluble compound, thereby reducing absorption and interrupting enterohepatic cycling of thallium and increasing elimination (Malbrain *et al.*, 1997). Multiple doses of activated charcoal may provide similar benefit.

Forced diuresis also increases the elimination of thallium. Potassium chloride (KCl) increases thallium excretion by mobilizing thallium from tissues, but it must be used cautiously since it may aggravate toxicity as a result of the increased plasma

thallium concentration (Vrij, Cremers, and Lustermans, 1995). Haemodialysis and haemoperfusion may also increase thallium elimination (Malbrain *et al.*, 1997).

Yellow phosphorous

Lavage with potassium permanganate (1:5000) is recommended for yellow phosphorous ingestions. This is intended to convert phosphorous to relatively harmless phosphorous oxides. Activated charcoal adsorbs phosphorous and should be administered after lavage to limit the absorption of any remaining elemental phosphorous (Simon and Pickering, 1976). The remainder of treatment consists of symptomatic and supportive care.

Phosphides

The use of sodium bicarbonate or milk in an attempt to reduce gastric acidity following phosphide ingestion has been proposed as a means to slow the generation of phosphine gas (Cienke, 2001). Although there is a lack of supporting evidence that any of these methods are effective, they are worth trying since they are relatively benign therapies. Activated charcoal should be administered in these cases. The remainder of treatment consists of symptomatic and supportive care.

Barium

Barium does not bind to activated charcoal. Oral administration of either sodium or magnesium sulphate to form a precipitate of barium sulphate is recommended to reduce gastrointestinal absorption (Wetherill, Guarino, and Cox, 1981). The remainder of treatment consists of symptomatic and supportive care, including frequent monitoring of serum potassium and aggressive potassium replacement therapy.

Cholecalciferol

The use of repeated doses of activated charcoal has been suggested for cholecalciferol poisoning, but there are no clinical data to support this. Cholecalciferol-induced hypercalcaemia should be managed with traditional supportive measures, including diuresis, prednisone, calcitonin, or mithramycin in non-responsive cases.

Vacor

Standard gastrointestinal decontamination procedures should be followed for exposures to vacor. Intravenous niacinamide (nicotinamide) has been shown to be effective as an antidote in the rat and is most effective if administered within an

hour of exposure (Johnson, Kubie, and Levitt, 1980). Intravenous niacinamide is not available in the United States. While oral therapy with niacinamide is recommended for maintenance therapy in patients who develop evidence of vacor toxicity, there are no data on its effectiveness if administered in acutely poisoned patients. Given the relative benign nature of oral niacinamide, it seems reasonable to administer niacinamide orally if the intravenous form is not available. The remainder of therapy for vacor poisoning consists of symptomatic and supportive care, including insulin therapy to manage vacor-induced diabetes.

Red squill

Red squill has a powerful emetic action, which may help limit absorption following ingestion. Activated charcoal is indicated to limit absorption of any remaining substance in the gastrointestinal tract. Cardiotoxic effects of red squill are best managed in the same manner as the digitalis glycosides, including digoxin-specific antibody fragments.

Miscellaneous rodenticides

Therapy for poisoning due to bromethalin, sodium monofluoroacetate, ANTU, or Norbromide consists of symptomatic and supportive care. There are no specific therapies or antidotes for the treatment of poisonings due to these rodenticides.

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Part VI

Regulation

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13 Regulation of Pesticides and Biocides in the European Union

Deborah J. Hussey and **Graham M. Bell**

Background

The authorization of pesticides and biocides in the European Union is governed by two main Directives, Directive 91/414/EEC (EEC Council, 1991) concerning the placing of plant protection products on the market, and Directive 98/8/EC (European Parliament and EC Council, 1998) concerning the placing of biocidal products on the market. Where appropriate these Directives call on other European legislation in related areas such as test methods, classification and labelling, and maximum residue levels (MRLs).

Directive 91/414/EEC is primarily concerned with the regulation of agricultural pesticides. It defines plant protection products as chemical or biological products intended to: protect plants or plant products against harmful organisms; influence the life processes of plants, other than as a nutrient (e.g. growth regulators); preserve plant products; destroy undesired plants or parts of plants; and check or prevent undesired growth of plants.

Directive 98/8/EC defines biocidal products as chemical or biological products intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism. It establishes an exhaustive list of 23 product types, in four main areas, as follows:

1. Disinfectants and general biocidal products (human hygiene products; private area and public health area disinfectants and other products; veterinary hygiene products; food and feed area disinfectants; drinking water disinfectants).

2. Preservatives (in-can; film; wood; fibre, leather rubber and polymerized materials; masonry; slimicides; metalworking fluids).

3. Pest control products (rodenticides; avicides; molluscicides; piscicides; insecticides, acaricides and products to control other arthropods; repellents and attractants).

4. Other biocidal products (preservatives for food and feedstock; antifouling products; embalming and taxidermist fluids; products to control other vertebrates).

Prior to the introduction of Directives 91/414/EEC and 98/8/EC, the regulation of these products was the responsibility of the various Member States of the European Union. All Member States had their own national registration schemes for plant protection products. For biocidal products the situation was very different with some product types regulated in all Member States, others in none. Where products were not subject to a national registration scheme their supply was according to the general legislation on chemicals.

There is a potential overlap between Directives 91/414/EEC and 98/8/EC. Products that are exclusively intended to control organisms having a detrimental effect on plants or plant products, either if applied directly or indirectly on empty structures (e.g. a grain silo), are plant protection products. Those products that control organisms detrimental to humans or to other goods are biocidal products. For the control of mice, rats, or other rodents, products used in plant-growing areas (e.g. agricultural fields, greenhouses, forests) to protect plants or plant products, and products for the control of moles, are plant protection products. Those used outside plant-growing areas (e.g. on farms, cities, industrial premises) and in plant-growing areas for hygiene purposes are biocidal products. There is also a potential overlap between Directive 98/8/EC and other products, in particular medicines, veterinary medicines, and cosmetics. Products that are within the scope of Directives on Medicines, Veterinary Medicines, Cosmetics, etc. are outside the scope of Directive 98/8/EC.

Both Directives 91/414/EEC and 98/8/EC lay down harmonized procedures that must be followed by all Member States, and work by a two-phased procedure. In the first phase active substances are evaluated at European level and, if acceptable, are included in Annex I of the respective Directive. Only substances that can be shown to be used in products without significant risk to human or animal health or to the environment, on the basis of a comprehensive data package, will be included in Annex I. Once an active substance has been included in Annex I it may then be incorporated into products for use within the European Union. Such products must be authorized within each Member State in which they are to be used according to common rules that are described later.

When these Directives came into force (91/414/EEC in July 1993 and 98/8/EC in May 2000) they had no immediate effect on the supply of existing active substances and products. Supply of these can continue (in accordance with national rules, if any) until the active substances have been reviewed (a process that the Directives indicated should be completed in 10 years) and a decision made whether

they can be included in Annex I. Products based on a new active substance (i.e. an active substance not used in products before the respective Directive came into force) cannot be marketed until the active substance has been included in Annex I.

The review programmes for existing active substances are established by means of a series of EC Regulations made under the respective Directive. The principles adopted by both Directives are essentially the same, but the timings differ. Both Directives aim to review high priority active substances first and ensure that unsupported active substances are withdrawn from the marketplace.

Under Directive 91/414/EEC, a list of 90 active substances for review in the first phase of the programme was established in Regulation 3600/92/EC (EEC Commission, 1992). This Regulation also set dates by which companies, or applicants, must submit dossiers of data for assessment and established to which rapporteur Member State these should be sent. It was originally intended that further lists of approximately 90 active substances would be published annually until the review programme was complete (within the 10-year period). However, as work has progressed much more slowly than originally envisaged, this has not been possible and the programme has been extended to 2008. A second review Regulation (451/2000/EC) (EC Commission, 2000a) has also been published. This did two things: firstly, it established a list of 148 active substances for review in the second phase, and the actions suppliers must take if they wished to support the active substance; secondly, it sets out what suppliers of 389 active substances in the third phase must do if they wished to support these in the review programme. Essentially, for both parts, suppliers had to notify their intention to support the active substance and provide a summary of all the studies required, and then provide the dossier of data to the rapporteur Member State at the appropriate time.

Dossiers for active substances in the second phase had to be submitted by April 2002 and those for the third phase must be provided in either November 2003 or November 2004. Further Review Regulations (703/2001/EC) (EC Commission, 2001b), (1490/2002/EC) (EC Commission, 2002a) set out the details for these two phases, including the rapporteur Member States.

Plant protection products containing active substances that are not supported will no longer be able to be marketed after July 2003. Active substances that have been supported can be used in plant protection products until a decision on Annex I listing has been made. Over 300 active substances in the second and third phases have not been supported and this will result in the loss of many products in 2003. The Commission has responded by granting a number of ‘essential uses’ that may continue to 2007 to allow alternatives to be developed.

The fourth and final phase of the review is set out in Regulation 1112/2002/EC (EC Commission, 2002b). This lists 238 active substances, including micro-organisms and plant extracts, and requires suppliers to notify their intent to support these in the review programme.

For Directive 98/8/EC, the first Review Regulation (1896/2000/EC) (EC Commission, 2000b) and the ‘Prolongation’ Regulation (1687/2002/EC) (EC Commission,

2002c) required that all suppliers intending to support an existing active substance notified this intention. Active substances that have been notified can continue to be used in biocidal products until a decision on Annex I inclusion has been made. Active substances that suppliers were not intending to support had to be identified and will no longer be able to be used in biocidal products after 1st September 2006. Active substances that have neither been identified nor notified cannot be used in biocidal products after autumn 2003. In addition to this, the first Review Regulation gave first priority to the review of active substances used in wood preservatives and rodenticides. The second Review Regulation, expected to be published in autumn 2003, will give the rapporteur Member State for individual active substances used in wood preservatives and rodenticides, together with further priority lists for review.

Data requirements

Both Directives contain Annexes giving brief details of the data required for active substances (both chemicals and micro-organisms) and for products containing them. These data requirements address 10 main areas: identity of the active substance/organism or product; physical and chemical properties/biological properties; analytical methods; efficacy data; toxicological studies; residues in or on treated products, food, and feed; fate and behaviour in the environment; ecotoxicological studies; classification and labelling proposals; and a summary and evaluation of the data provided.

It is important to note that both Directives allow applicants to submit a justification in place of data where they believe certain pieces of information would not be necessary owing to the nature of the active substance or to the proposed uses of products containing it. This is particularly the case for biocidal products, which can have very different uses and exposure profiles. Products can range from those having indoor use only to antifouling products with direct entry into the environment. Some products can be used only in industrial processes whilst others have widespread exposure to the general public.

The toxicological studies required for a chemical active substance include: acute toxicity (oral, percutaneous, inhalation, skin and eye irritation, skin sensitization); sub-chronic toxicity; genotoxicity testing; chronic toxicity and carcinogenicity; reproductive toxicity (both developmental and effects on fertility); neurotoxicity studies; metabolism in mammals; and available medical data. For a product containing a chemical active substance, the following are usually needed: acute toxicity; data on exposure through application (this includes the pattern of use); and dermal absorption.

For active substances and products based on micro-organisms, a tiered testing system has been developed and the toxicological data required also include pathogenicity and infectivity studies.

For Directive 91/414/EEC, further guidance on the data requirements and details of the test guidelines to be followed has been provided in a series of amending Directives. Directive 94/79/EC (EC Commission, 1994) gives the expanded details for toxicological and metabolism studies for chemical substances and plant protection products and Directive 2001/36/EC (EC Commission, 2001a) addresses micro-organisms and products containing them.

Owing to the wide-ranging scope of Directive 98/8/EC, the data requirements are separated into a 'common core' set for chemical active substances and products containing them, with 'additional' requirements specified in certain situations. For example, in some situations it may be possible to justify not submitting data on chronic toxicity and carcinogenicity, some of the sub-chronic and reproductive toxicity studies and neurotoxicity. For Directive 98/8/EC, the Commission has produced draft Technical Notes for Guidance on data requirements for active substances and biocidal products. Whilst the data requirements for active substances based on microbiologicals and their products are outlined in Directive 98/8/EC, further guidance is currently not available.

Tests should be undertaken using methods recognized and recommended by competent international bodies wherever possible. Consequently, the majority of test guidelines referred to in the guidance documents are for methods from the European Plant Protection Organisation (EPPO) or given in the Dangerous Substances Directive (67/548/EEC) (EEC Council, 1967). Many of the latter methods have been adopted from those of other international organizations, such as the Organization for Economic Co-operation and Development (OECD).

The toxicological and other information provided must be sufficient to permit an evaluation to be made as to the risks for humans. The risks will include those associated with the handling and use of products through to disposal, and those associated with secondary exposure (including residues in food and water, on surfaces, volatilized around the home and in workplaces). In addition and when appropriate, the information provided must be sufficient to establish levels such as: the no observed adverse effect level (NOAEL); the acceptable operator exposure level (AOEL); the acceptable daily intake level (ADI) for humans; and the maximum residue levels (MRLs) in treated food crops.

Evaluation and decision-making process

Annex I inclusion

A company, or applicant, wishing to market a plant protection or biocidal product must first secure inclusion of the active substance in Annex I. The applicant must submit an appropriate dossier or data package. This will include complete data sets for the active substance, and for at least one representative product containing it. The applicant must also provide a summary and thorough evaluation of the

information in the dossier. The dossier must be prepared to a standard format, as detailed in Commission guidelines, and should preferably be submitted in the recommended electronic format.

Once a dossier has been verified as complete, the appointed rapporteur Member State then has 12 months in which to produce a 'monograph' or 'draft assessment report' (Directive 91/414/EEC) or 'evaluation' or 'competent authority report' (Directive 98/8/EC), again prepared in accordance with Commission guidelines. The structure of the Member State's draft assessment report or competent authority report reflects the format of the company dossier and will include a risk assessment and a recommendation for inclusion or non-inclusion of the active substance in Annex I. Rapporteur Member States working under Directive 98/8/EC will peer-review the applicant's evaluation of the hazard information and provide a commentary, rather than drafting a separate report. This approach is designed to increase transparency in the decision-making process and improve throughput. The rapporteur will produce the risk assessment.

The rapporteur's draft assessment report or competent authority report is then subject to review by representatives from all Member States. The final decision on the inclusion or not of an active substance in Annex I normally will be taken by the Commission, after obtaining a favourable opinion from the Standing Committee on the Food Chain and Animal Health (SCFA) or the Standing Committee on Biocides (SCB). This opinion is delivered by the Member States in the SCFA/SCB and is reached by qualified majority voting. For Directive 91/414/EEC, conditions attached to the Annex I inclusion of substances are published as Commission Directives. In addition, detailed reports containing the draft assessment report as a background document are made available by Member States for consultation by any interested party. These processes have yet to be finalized for biocides. The inclusion of an active substance in Annex I can be renewed on one or more occasions for periods not exceeding 10 years. The Annex I inclusion can also be reviewed at any time if there are concerns that the substance presents a risk to health or the environment.

As with any new programme of this complexity, the first examples through the system will always take longer to process while procedures are established and problems resolved. In order to speed up the evaluation process under Directive 91/414/EEC, the Commission has established procedures such as: peer review of draft assessment reports by several Member States prior to their consideration by the SCFA; and the appointment of co-rapporteurs to assist the rapporteur Member State in the preparation of the draft assessment report. Such co-operation between Member States has also led to increased confidence in each other's decision-making. Similar collaborative measures are likely to operate under Directive 98/8/EC.

There are some important differences in the structure and operation of the two Directives. Directive 98/8/EC provides for two additional lists, Annexes IA and IB. Annex IA is for active substances that may be included in low-risk products. These products have data requirements and processing time-scales that are reduced compared

with those for other biocidal products. Annex IB is for basic substances. These are substances (e.g. carbon dioxide, ethanol) whose major use is non-pesticidal but which have a minor use as a biocide – a use for which they are not directly marketed.

In addition, Directive 98/8/EC allows for 'comparative assessment' whereby, in certain circumstances, the entry of an active substance on Annex I may be refused or removed if another substance presents significantly less risk to health or the environment under normal conditions of use. The process must ensure that there remains adequate chemical diversity of active substances to minimize the occurrence of resistance in target organisms and should only be applied to products in the same product type. Also, there should be the opportunity of acquiring experience from use before a decision is taken.

Product authorization

Once an active substance has been included in Annex I, Member States must assess products containing it prior to issuing a national authorization (or registration). This assessment will ensure that there is compliance with any conditions attached to the Annex I entry, and with the data requirements and rules on data protection. To ensure a consistent approach, Member States have agreed rules that must be observed when authorizing products, namely the Uniform Principles (published as a separate Directive for plant protection products (EC Council, 1997)) or Common Principles (for biocidal products). These Principles encompass evaluation and decision-making for the authorization of products in areas such as: effects on humans; effects on animals; effects on the environment (water, soil, air, non-target organisms); effects on plants; and efficacy. Differences between the use patterns of plant protection and biocidal products are reflected in the respective Principles. For example, plant protection products can remain as residues in or on treated products, food, or feed. The Uniform Principles therefore include analytical methods and require the estimation of the potential exposure of consumers through the diet, and if applicable other routes, using a suitable calculation model.

Once a product has been authorized (or registered) in one Member State, both Directives allow for it to be mutually recognized in another, should a company or applicant so request. A Member State would not be allowed to refuse to authorize a plant protection product already registered in another Member State if all agricultural, plant health, and environmental considerations relevant to the use of the product were comparable. For biocidal products the same principle applies and authorization cannot be refused unless target species, resistance/tolerance, or other relevant circumstances of use (such as breeding period of target species or climate) differ significantly.

Products containing new active substances

Member States are allowed to issue provisional authorizations for products containing a new active substance, once the dossier has been verified as complete and

provided the Member State believes that the requirements of the Directives can be met. Provisional authorization can be granted for 3 years or more to allow companies to market these new products in individual Member States while consultations for Annex I inclusion progress.

Research and development and emergency authorizations

Both Directives make provisions for use of unauthorised products in research and development (R&D) and in emergency situations. In both Directives there is a requirement to obtain an authorization before any experiment or test involving the release into the environment of unauthorized products or non-Annex I listed active substances is carried out. This will only be allowed under controlled conditions and for limited quantities and areas. Directive 98/8/EC also places restrictions on other aspects of R&D and process-orientated research and development (PORD). These restrictions, and the definitions of R&D and PORD, are taken from the Dangerous Substances Directive (67/548/EEC).

Member States may also authorize a plant protection or biocidal product for a period of up to 120 days for limited or controlled use if such a measure appears necessary because of unforeseeable danger that cannot be contained by other means.

Progress with implementation of the Directives

Plant protection products

To date, decisions have been made under Directive 91/414/EEC to include over 70 new or existing active substances on Annex I, while 24 existing active substances have not been recommended for Annex I inclusion. Experience and policy, technical and procedural developments since 1991 have demonstrated the need for changes to Directive 91/414/EEC in a number of key areas. Negotiations on a major revision of the Directive are due to begin towards the end of 2003. The proposals to be considered are likely to include: new provisions for data protection and data-sharing; fast track procedures for low-risk products; comparative assessment; enhanced transparency, and greater public participation in the authorization process; and zonal authorizations covering more than one Member State.

Biocides

Since the date of implementation of Directive 98/8/EC on 14 May 2000 there have been no applications for the inclusion in Annex I of new active substances. The closing date for submitting identifications or notifications under the first Review

Regulation (1896/2000) was 28 March 2002 and notifications under the 'Prolongation' Regulation was 31 January 2003.

The final draft of the second Review Regulation lists 955 existing active substances as having been identified. Of these, 363 have been acceptably notified and will be reviewed for possible inclusion in Annex I.

The second Review Regulation also:

1. Details the obligations of applicants, Member States and the Commission and the procedures for the review of active substances.

2. Confirms the date for submission of full dossiers on active substances used in wood preservatives and rodenticides as 28 March 2004 and also lists the rapporteur Member States.

3. Lists the rapporteur Member States for active substances on the second priority list. These are active substances used in molluscicides; insecticides, acaricides and products to control other arthropods; repellents and attractants; and antifouling products. Full dossiers must be submitted by 30 April 2006.

4. Gives the submission deadlines for the third and fourth priority lists and the product types to be reviewed (List 3 – full dossier to be submitted by 31 July 2007 – human hygiene biocidal products; private area and public health area disinfectants and other biocidal products; veterinary hygiene biocidal products; food and feed area disinfectants; drinking water disinfectants; in-can preservatives; metalworking-fluid preservatives. List 4 – full dossiers to be submitted by 31 October 2008 – film preservatives; fibre, leather, rubber, and polymerized materials preservatives; masonry preservatives; preservatives for liquid-cooling and processing systems; slimicides, avicides; piscicides; preservatives for food or feedstocks; embalming and taxidermist fluids; products for the control of other vertebrates).

Classification and labelling

The Dangerous Substances Directive (67/548/EEC), as amended, regulates the classification and labelling of substances that present a hazard to humans and the environment. The Directive defines substances as 'dangerous' if they are explosive, oxidizing, flammable, toxic, harmful, corrosive, irritant, sensitizing, carcinogenic, mutagenic, toxic for reproduction, or dangerous to the environment. Directive 67/548/EEC describes the test methods to be used for assessing these properties, together with the relevant classification criteria and the associated labelling requirements (hazard symbols and risk and safety phrases). There is a related Directive,

recently revised as 1999/45/EC (European Parliament and EC Council, 1999), that regulates the classification and labelling of most dangerous preparations, including plant protection products and biocides. This Directive provides calculation methods for assessing the classification of a product (from the hazard properties of its components) where test data on the product are not available.

Both Directives 91/414/EEC and 98/8/EC include extensive labelling requirements for plant protection and biocidal products, respectively. Under Directive 91/414/EEC these include directions for use (including any special agricultural, plant health, or environmental conditions under which the product should not be used) and safety intervals (such as harvesting and re-entry intervals). Plant protection products classified and labelled for hazard in accordance with Directive 1999/45/EC should also carry the phrase: 'To avoid risks to man and the environment, comply with the instructions for use.' Similarly, directions for use are included under Directive 98/8/EC, together with information on, for example, time intervals between applications, re-entry intervals, ventilation of treated areas, decontamination methods, and cleaning of equipment.

Maximum residue levels (MRLs)

Legislation on maximum residue levels (MRLs) in food and agricultural commodities is currently covered by four separate Council Directives: 76/895/EEC (fruit and vegetables) (EEC Council, 1976); 86/362/EEC (cereals) (EEC Council, 1986a); 86/363/EEC (foodstuffs of animal origin) (EEC Council, 1986b); and 90/642/EEC (a wider range of products of plant origin, including fruit and vegetables) (EEC Council, 1990). MRLs are designed to ensure that users of pesticides comply with good agricultural practice (GAP) and are intended primarily for trade purposes. The MRL Directives place obligations on Member States to monitor treated produce for residues of pesticides as a key mechanism to ensure compliance.

The establishment of MRLs is also an integral part of the authorization process under Directive 91/414/EEC. Under the current system, when a new active substance is added to Annex I of Directive 91/414/EEC, a linked Directive on MRLs is also introduced. Such Directives establish substantive limits for the approved uses and set MRLs for all other crops at the limit of determination (LOD). All these new limits are viewed as 'provisional' (for a period of 4 years) in the sense that there should be quick changes to the legislation if new uses or new conditions of use (leading to higher residues) are authorized. A decision not to include an active substance in Annex I of Directive 91/414/EEC is followed up by a Directive setting MRLs at the LOD, unless a specific import tolerance (to take account of imports from outside Europe) has been granted in any particular case.

At the time of writing the Commission is preparing a draft proposal consolidating the four original MRL Directives and recasting and simplifying the existing legislation.

Harmonization

Technical harmonisation within the European Union has been achieved through the development of detailed data requirements for active substances and plant protection or biocidal products, the detailed evaluation and decision-making principles for such products (Uniform or Common Principles) and the guidelines concerning the presentation of dossiers and assessment reports or evaluations. The European dossier and monograph guidelines for Directive 91/414/EEC have formed the basis for similar guidance that has been developed at OECD level. This can be seen as an important step towards harmonization and work-sharing in the evaluation of pesticides on a wider international scale. For biocides the OECD has identified harmonization of data requirements, test methods, and exposure and risk assessment methodologies as important areas of work. Current priorities are environmental exposure assessment and efficacy data requirements and test methods.

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14 Regulation under NAFTA

Cheryl E. A. Chaffey and **Virginia A. Dobozy**

History and legislation

The United States

The intent and focus of pesticide regulation in the United States has evolved dramatically since the first federal food legislation, the Pure Food and Drug Act, was passed in 1906. This law guaranteed the wholesomeness and truthfulness of labelling of foods, drugs, and cosmetics (USA, 1906). It was followed by the first specific federal pesticide legislation, the Insecticide Act of 1910 (USA, 1910). Administered by the US Department of Agriculture (USDA), it was intended to prevent the manufacture, sale, or transportation of impure or improperly labelled insecticides and fungicides. In 1938, food legislation was updated with the passage of the Federal Food, Drug and Cosmetic Act (FFDCA), which added the authority for establishment of regulatory limits of pesticides and other harmful substances in food (USA, 1938). A more comprehensive pesticide law, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) was passed in 1947 (USA, 1947). It required that all pesticides, including herbicides and rodenticides, sold interstate be registered with the USDA; however, it did allow registrations to go forward over USDA's 'protest' and the Agency had to take affirmative action to cancel such registrations. In 1954, the Miller amendment to FFDCA required that tolerances (maximum legal residue concentrations) be established for pesticide residues on food and animal feed. A controversial and complex amendment to FFDCA was passed by Congress in 1958. Commonly known as the Delaney Clause, it prohibited the use of any food additive that was shown to cause cancer in humans or laboratory animals. Pesticide residues in processed foods at levels higher than in the raw agricultural commodity (RAC) were subject to this legislation, but pesticides that did not concentrate in processed foods were not subject to the Delaney Clause. The publication of *Silent Spring* by Rachel Carson in 1962, which warned about the dangers of organochlorine pesticides, ignited public interest in environmental issues (Carson, 1962). In 1964, the FIFRA was amended to remove 'protest' registrations. In 1970, major regulatory shifts occurred when FIFRA administration was transferred to the US Environmental Protection Agency (USEPA), a new federal agency. Tolerance

establishment was transferred from the Food and Drug Administration (FDA) to USEPA, but the authority for tolerance enforcement remained with FDA.

A comprehensive overhaul of the FIFRA, passed in 1972, required scientific evidence that a pesticide would present ‘no unreasonable adverse effect’ to humans and the environment. The re-registration of pesticides registered prior to 1984 was the focus of the 1988 FIFRA amendments. The USEPA was required to perform accelerated comprehensive re-evaluations of these pesticides and ensure that they measured up to current scientific and regulatory standards. In 1993, the USEPA established a policy to grant priority review to low-risk pesticides in an effort to register pesticides with less potential to cause adverse health and environmental effects.

On 3 August 1996, the Food Quality Protection Act (FQPA) amended the FFDCA to create a single, health-based standard for pesticides in food (US, 1996). The safety standard was revised from ‘protect public health’ to ‘reasonable certainty of no harm’. This standard was also incorporated into the FIFRA for food-use pesticides. By creating a single standard, the inconsistencies between allowed residues on processed foods and RACs from the Delaney Clause were eliminated. The FQPA required that all existing tolerances be reviewed within 10 years to make sure they meet the new health-based safety standards. In establishing tolerances, the USEPA was required to provide for the protection of infants and children. The FQPA required the risk from all non-occupational sources of pesticide exposure, i.e. food, drinking water, and household uses (aggregate risk assessment) to be assessed. In addition, the risks of chemicals with a common mechanism of toxicity must be combined (cumulative risk assessment). The FQPA also required the potential effects of pesticides on the endocrine system to be assessed.

Canada

In the early 1900s, pesticides in Canada were first regulated as poisons by the Department of Health and dispensed by registered pharmacists. With the expanding use of agricultural chemicals, the Agricultural Pests’ Control Act came into force in 1927 administered by the Department of Agriculture. While establishing a pre-market registration system and introducing labelling requirements, the Act did not require analysis of risk or efficacy. In 1939 the Act was revisited and among other things required data pertaining to the usefulness, effectiveness, and safety of the product. With heightening concerns about environmental impact and the emergence of more complex synthetic pesticides, the Pest Control Products Act (PCPA) was passed by Parliament in 1969. The PCPA was brought into force by the adoption of Regulations in 1972 (Canada, 1985a) following negotiations that forged a relationship between federal and provincial authorities. Departments of Health, Environment, and Natural Resources (Forestry, Fisheries) served in an advisory capacity to the Department of Agriculture.

Following an extensive review of the pesticide regulatory system in 1990 by a group of multi-stakeholders, reform was undertaken, and in 1995, authority for the

administration of the PCPA was transferred from the Department of Agriculture to that of Health. Concurrently, the Pest Management Regulatory Agency (PMRA), the Canadian counterpart to the USEPA's Office of Pesticide Programs (OPP), was established within Health Canada. The expertise and functions from the participating departments (Agriculture, Health, Environment, and Natural Resources) were centralized in the PMRA, with the exception of research capability which was retained by the participating departments. The PCPA is the principal legislative tool that the PMRA utilizes in ensuring safety, merit, and value of pesticides through pre-market assessment or re-evaluation.

Also of importance is the Food and Drugs Act. This Act, originally promulgated in 1920, was the first Canadian legislation dealing with food adulteration and has resided with the Department of Health since its inception. Last revised in 1964 (Canada, 1985b), the Act and its accompanying Regulations are essential to the PMRA for promulgating maximum residue limits for pesticides on food and to the Canadian Food Inspection Agency (CFIA) for compliance and enforcement capability in ensuring a safe food supply. Additional legislation impacting on pesticide regulation can be found at the federal, provincial, and municipal levels.

NAFTA and harmonizing the approach to pesticide regulation

In 1992, the North American Free Trade Agreement (NAFTA) was signed by the US, Canada, and Mexico. Under the auspices of this agreement, the NAFTA Technical Working Group on Pesticides was formed in 1996 to develop a coordinated pesticides regulatory framework among NAFTA partners. Commitments were made to (1) share the work of regulation, (2) harmonize scientific and policy considerations, and (3) reduce trade barriers. Periodic meetings are held to monitor the progress and guide efforts on these commitments. Regular liaison between countries continues with a focus on resolving regulatory differences. One of the activities outlined in the North American Initiative for Pesticides (1998) was to harmonize risk assessment. This has been essential for the sharing of work which has become routine, particularly for the review of new products. Owing to the focus on harmonization, the passage of the FQPA in the United States has precipitated policy development and changes in approach to risk assessment in both the US and Canada. Much of the text below relates to these two countries, but will eventually apply also to Mexico.

Harmonization between the United States and Canada is facilitated by the fact that the respective definitions for a pesticide are similar. The FIFRA (US) definition is (1) any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest and (2) any substance or mixture of substances intended for use as a plant regulator, defoliant, or dessicant. The PCPA (Canada) defines pesticide as

any product, device, organism, substance or thing that is manufactured, represented, sold, or used as a means for directly or indirectly controlling, preventing, destroying, mitigating, attracting, or repelling any pest. Consequently, both regulatory agencies handle a wide range of products including but not limited to, herbicides, fungicides, insecticides, rodenticides, and biocides in a similar manner.

Data requirements

In both countries, new active ingredients must undergo extensive pre-market assessment. Applications to register a new active ingredient and its end-use formulations are submitted to the regulatory agencies with a comprehensive dossier of information and relevant fees. These dossiers are initially screened to ensure the completeness of the information for evaluation purposes and in the United States, are assessed against reduced risk criteria to establish review priority. Essential elements of the dossier include labelling and product chemistry information, human and environmental safety data, and in Canada, data to demonstrate efficacy and value. In the United States, efficacy data are only required for public health uses of pesticides such as in the control of bacteria, viruses, mosquitoes, ticks, cockroaches, fleas, rats, and mice.

Studies contained in the dossier are typically performed by the applicant's own laboratories or contract laboratories. They should comply with the principles of good laboratory practice (GLP), have adequate quality control and assurance, and be able to withstand the scrutiny of GLP audit. Typically, these studies must contain all the details of the scientific study. Scientific publications are used as supplemental data but cannot fully replace the comprehensive data submitted in support of product regulation. It is the responsibility of chemists, biologists, toxicologists, agronomists, entomologists, and others in the regulatory agencies to perform a detailed evaluation of the studies to gain a thorough understanding of the risks and benefits associated with any given pesticide.

Product chemistry information is required on the technical active ingredient and formulated products regarding the identification and quantification of all technical active ingredients, impurities and formulants, description of manufacturing or formulating processes, analytical methodologies, and chemical and physical properties. Particular attention is given to the quantification of toxic impurities or byproducts, and products suspected of having such contamination may require analysis at the limit of detection or quantitation for these contaminants (e.g. dioxins, nitrosamines). Wherever it is possible for a product to enter the environment, information is required on the fate (e.g. persistence, potential transformation products) of the technical active ingredient in various media (e.g. soil, air, water). Toxicity testing of the technical active ingredient and any relevant transformation products in various species reflecting the diversity of potentially exposed ecosystems (e.g. aquatic plants, beneficial insects, birds) is required and assessed along with the environmental fate data to provide an assessment of environmental risk.

The remainder of the chapter is focused on the characterization of human risk from pesticide exposure.

Hazard identification

The identification of human health hazards associated with a technical active ingredient and its end-use formulations is of paramount importance in both American and Canadian regulatory regimes and is crucial for the characterization of risk that may result from the use of or exposure to the pesticide in question. Extensive and costly toxicological testing is conducted in laboratory animals or cell cultures according to well-established guidelines such as those of the USEPA or Organization for Economic Co-operation and Development (OECD). The most comprehensive toxicology data are required for traditional agricultural chemical pesticides and the requirements are similar in both the United States and Canada (Table 14.1). Data requirements for other types of products, such as microbials and pheremones, are generally not as extensive as those for agricultural chemicals given the nature of these products and are also generally harmonized between countries. Both countries allow for certain data requirements to be waived if adequate scientific justification is provided by the applicant.

In a typical database, acute studies are required primarily for classification and labelling, although additional information can be gleaned from these studies such as identification of symptoms of acute poisoning, contribution of formulants to the toxicity profile, etc. Toxicokinetic studies provide information on the absorption, distribution, metabolism, and excretion of a compound and typically address these parameters comparing low and high dosages as well as single and repeat exposures. Particular attention is given to whether the compound bioaccumulates or undergoes biotransformation to more toxic entities. Short-term, repeat-dose studies have numerous uses including the identification of target organ toxicity, species differences, route differences, effect of duration on dosing (i.e. cumulative action), and when conducted with a withdrawal subgroup, can provide valuable information on reversibility. Lifetime studies elucidate the potential for chronic toxicity and carcinogenicity of a compound. Carcinogenicity studies are complemented by a battery of genotoxicity studies that assess the potential effects of a compound on the gene or chromosome.

Multi-generation reproduction studies are crucial in assessing potential effects on reproductive capacity and resulting offspring. These studies allow for the detection of functional toxicity, such as subsequent reproductive performance, following *in utero* as well as direct exposure. Developmental studies examine a product's potential to induce congenital malformations, alter other developmental parameters, and exert a toxic influence on a pregnant animal. The multi-generation reproduction and developmental studies are primary indicators for identifying potential sensitivity of the young as required by both the EPA and the PMRA. These studies are

Table 14.1 Toxicity studies that are typically required (TR) or conditionally required (CR) for a conventional food-use pesticide

Study type	Test material	Route	Preferred species
Acute toxicity – TR	TGAI, EUP	Oral, dermal, inhalation	Rat
Acute irritation – TR	TGAI, EUP	Eye, dermal	Rabbit
Sensitization – TR	TGAI, EUP	Dermal	Guinea pig
Sub-chronic toxicity – TR	TGAI	Oral	Rat, dog
	TGAI	Dermal	Rat or rabbit
	TGAI	Inhalation[a]	Rat
Carcinogenicity – TR	TGAI	Oral	Rat, mouse
Chronic toxicity – TR	TGAI	Oral	Rat
Reproductive toxicity – TR	TGAI	Oral	Rat
Developmental toxicity – TR	TGAI	Oral	Rat, rabbit
Genotoxicity – TR	TGAI	*In vivo* and *in vitro* assays	
Toxicokinetics/metabolism – TR	TGAI	Oral	Rat
Acute neurotoxicity – CR	TGAI	Oral	Rat
Short-term neurotoxicity – CR	TGAI	Oral	Rat
Developmental neurotoxicity – CR	TGAI	Oral	Rat
Delayed neurotoxicity – CR	TGAI	Oral	Hen
Immunotoxicity – CR	TGAI	Oral	Rat

TGAI = technical grade active ingredient.
EUP = end use product.
[a]Required if exposure by inhalation route expected.

carefully evaluated to determine if young animals are qualitatively and/or quantitatively affected more than their parents. In the last several years, the emphasis on protection of the young in both regulatory agencies has resulted in more rigorous comparisons of toxicity in adult compared with prenatal/neonatal organisms.

For compounds demonstrated to have neurotoxic properties in standard toxicity studies, such as organophosphates, neurotoxicity studies play a major role. In addition to a delayed neurotoxicity study, additional acute and short-term neurotoxicity studies with detailed neurobehavioural and neuropathological assessment are required. Dependent on the toxicity profile observed, a developmental neurotoxicity study may also be required. The potential for immunotoxicity can be identified through the measurement of relevant parameters in already required studies, which in turn could trigger a requirement for specific immunotoxicity studies. Likewise, the potential for endocrine modulation is currently assessed from existing studies, but more refined assessment tools are under development and ultimately will be reflected in regulatory requirements.

Dietary risk assessment

The USEPA and the PMRA typically assess the risk of pesticide exposure in food on both an acute (single day) and chronic (long-term) basis. For acute dietary risk, the no observed adverse effect level (NOAEL), the highest dose at which no adverse effects were observed, from appropriate animal toxicity studies is divided by appropriate uncertainty factors. The endpoints typically chosen in this assessment are those that either result from a single-dose study (e.g. clinical signs in an acute neurotoxicity study) or although detected in a repeat-dose study, could possibly be induced by a single exposure (e.g. malformations in a developmental study). If no relevant endpoint is identified, then an acute dietary risk assessment is not necessary in either country. In the United States, the resulting value, known as the acute reference dose (aRfD) is further divided by a FQPA safety factor in the event that the compound shows evidence of sensitivity to the young. This revised value is referred to as an acute population adjusted dose (aPAD). In Canada, uncertainty factors and additional safety factors for sensitivity of the young or for severity of endpoint are incorporated into the acute reference dose (ARD) calculation in a one-step process. Consequently, the US aPAD or its Canadian equivalent, the ARD, is considered to be the level of 1-day exposure to a pesticide residue that is believed to have no significant deleterious effect in humans, including children. For the chronic dietary risk, a similar approach is employed. The NOAEL, typically selected from a repeat-dose study and reflecting an endpoint usually associated with prolonged or continuous exposure, is divided by appropriate uncertainty factors. In the United States, the resulting value, known as the chronic reference dose (cRfD), is divided by the FQPA safety factor as appropriate which becomes a chronic population adjusted dose (cPAD). The Canadian equivalent to the cPAD, known as the acceptable daily intake (ADI), is also derived taking into account uncertainty and safety factors. This value is believed to be the level of dietary exposure to a pesticide residue that, over a lifetime, is considered to have no significant harmful effects in humans, including sensitive sub-populations such as children (Table 14.2).

The excess risk of cancer resulting from dietary exposure to pesticides determined to be potential carcinogens is also assessed. For carcinogenic effects thought to occur through a non-threshold mechanism, a computer model is used for low-dose extrapolation of the animal data to generate a cancer potency factor known as q_1^*. The q_1^* can be thought of as the slope of the dose–response curve; compounds with high q_1^* values are considered more potent carcinogens. In cases where extensive mechanistic data are available to support a threshold mechanism, a point of departure (PoD) is derived that marks the beginning of the carcinogenic response. The PoD can be either a NOAEL, a lowest observed adverse effect level (LOAEL), or an interpolated value such as a benchmark dose.

As dietary risk is a function of exposure and the toxicity of the chemical, estimates of dietary exposure are required for all food-use pesticides. Dietary exposure from food

Table 14.2 Derivation of dietary reference doses

US	Canada
$\text{aRfD} = \dfrac{\text{NOAEL}}{\text{uncertainty factors}}$	
$\text{aPAD} = \dfrac{\text{aRfD}}{\text{safety factor (unique to FQPA)}}$	$\text{ARD} = \dfrac{\text{NOAEL}}{\text{uncertainty} \times \text{safety factors}}$
$\text{cRfD} = \dfrac{\text{NOAEL}}{\text{uncertainty factors}}$	
$\text{cPAD} = \dfrac{\text{cRfD}}{\text{safety factor (unique to FQPA)}}$	$\text{ADI} = \dfrac{\text{NOAEL}}{\text{uncertainty} \times \text{safety factors}}$

Uncertainty factors typically include a 10-fold factor to account for potential variation within the human population (intraspecies) and a 10-fold factor to account for potential differences between humans and animals (interspecies). Additional uncertainty factors of 3–10-fold may be applied where warranted (i.e. when a key study is missing or if a LOAEL, the lowest dose at which adverse effects were observed, is used instead of a NOAEL).

is determined from the amount of a pesticide residue that is present in and on food and the type and amount of food consumed. In a first-tier dietary exposure assessment in both the United States and Canada, residue values obtained from crop field trials conducted at maximum application rates and frequencies, minimum pre-harvest intervals with the assumption that 100 per cent of the crop is treated with the pesticide are used in the calculation. This yields tolerance values (US) or maximum residue levels (Canada) that will typically be used for compliance and enforcement purposes. For risk assessment purposes, additional data can result in more refined residue assessments. Refinements can include data on monitoring, percentage of crop treated, residue decline and degradation, effect of commercial or consumer processing (e.g. washing, cooking, peeling), and market basket data (actual residues on food at the time of purchase by the consumer) (USEPA, 2000a).

Food consumption data currently used by both countries are obtained from the USDA Continuing Surveys of Food Intake (USDA, 1996). In this survey, both 3-day mean consumption and single-day consumption information were recorded for 22 demographic and socio-economic sub-populations including infants, children, and nursing women. Further data from a Children's Supplemental Survey in 1998, once verified, will be incorporated into the risk assessments in both countries.

Both the United States and Canada use sophisticated software to combine food exposure data (residue levels, percentage of crop treated, and food consumption) with toxicity (reference doses) to produce a risk value. Acute food risk is expressed as a percentage of the aPAD (US) or ARD (Canada). If the calculated percentage aPAD or ARD is less than 100, the risk is generally considered to be acceptable (100 represents a full 'risk cup'). Acute exposure calculations tend to

include high-end residue values, high-end food consumption, and high-end percentage of crop treated estimates to reflect peak exposures that could be encountered as a single event. The software is capable of performing probabilistic analysis of these data for determination of acute risk and both agencies use risk estimates generated at the 99.9 percentile of probability where data on residue values in food are highly refined. Using the Monte Carlo approach, repeated sampling from the complete distribution of food consumption and the entire spectrum of pesticide residue data are used to predict the amount of pesticide likely to be consumed on a given day. Chronic dietary risk is calculated similar to the acute dietary risk with acceptable risk values below 100 per cent. For exposure estimates for chronic dietary risk, calculations typically include average residue values, average consumption values, and average percentage of crop treated.

$$\%\text{aPAD in the US or ARD in Canada} = \text{food exposure } (\text{mg kg}^{-1}\text{ day}^{-1})/\text{aPAD in the US or ARD in Canada} \times 100$$

$$\%\text{cPAD in the US or ADI in Canada} = \text{food exposure } (\text{mg kg}^{-1}\text{ day}^{-1})/\text{cPAD in the US or ADI in Canada} \times 100$$

For non-threshold carcinogens, cancer risk from food is calculated by multiplying cancer potency by average food exposure. This yields an upper bound probability of cancer risk (i.e. a calculated risk of 1×10^{-6} means that an individual receiving a lifetime exposure to the pesticide increases his/her chance of developing cancer by one in a million). Generally, dietary cancer risks that are in the neighbourhood of 10^{-4} to 10^{-6} are areas of regulatory concern.

$$\text{dietary cancer risk} = \text{average food exposure } (\text{mg kg}^{-1}\text{ day}^{-1}) \times q_1^* \ (\text{mg kg}^{-1}\text{ day}^{-1})$$

For threshold carcinogens, cancer risk from food is calculated as a ratio of the PoD for the tumorigenic response (e.g. NOAEL) and the average food exposure to yield a margin of exposure (MOE). Both countries are developing criteria to determine an appropriate target MOE.

Occupational/bystander risk assessment

Dependent on the use pattern for the pesticide, both the USEPA and the PMRA require occupational and residential risk assessments. Exposure monitoring studies

may be conducted to fulfil data requirements for the registration or re-registration of specific pesticides. In occupational studies, worker exposure is monitored under actual conditions of use for that pesticide. Two methods of quantifying exposure in these studies are passive dosimetry and biological monitoring. With passive dosimetry, the amount of pesticide in contact with clothing, skin, and/or breathing zone and thus available for systemic absorption is measured. With biological monitoring, the actual absorbed dose is estimated via measurements of pesticide and/or its metabolites in blood, urine, or exhaled air. This latter technique provides a more accurate measure of the absorbed dose but does not differentiate route of exposure. Surrogate data may also be used to quantify exposure provided that pesticides have similar attributes (e.g. formulation types, vapour pressure) and similar uses (e.g. rate of application, application equipment). The USEPA, the PMRA, and the California Department of Pesticide Regulation in co-operation with the pesticide industry, have developed a generic database, the Pesticide Handler's Exposure Database (PHED), which includes the results of over 100 exposure studies. The data can be sorted by criteria, such as formulation type, application method, clothing, and protective equipment to generate exposure estimates for similar occupational scenarios.

Assessing residential exposure is more complex owing to the large variety of possible exposure scenarios. Exposure is not only affected by the type of product used and the physical/chemical nature of the product, as with occupational exposures, but also by the nature of the human activities. Whereas worker exposure scenarios are fairly standard, the diversity of activities possible both indoors and outdoors in a residential setting is immense. Currently, both agencies use the Draft Standard Operating Procedures (SOPs) for Residential Exposure Assessments as a guidance. These SOPs identify approximately 15 common pesticide-related use patterns that result in residential exposure. Each of these is further divided into handler and post-application scenarios, which are then sub-divided by age group, route, and specific activity. The SOPs recommend the most appropriate means of assessing exposure and ensure a standardized approach for the variable scenarios.

After the human exposure estimates are obtained, they are compared with the most relevant endpoints from the animal toxicology studies for risk assessment. Selection of toxicology endpoints is based on the duration of exposure, route of exposure, and relevance of the toxicity endpoint to the population exposed. The most relevant NOAEL is divided by the exposure estimate to calculate a MOE. The MOE provides an estimate of how close the human exposure is to the NOAEL from the most relevant animal toxicity study. Thus, the MOE obtained from using the human exposure estimates is compared with a desired or target MOE. The desired or target MOE is influenced by the severity of the toxicity endpoint selected, and the completeness of the database. In the case of the residential risk assessment, the sensitivity of the exposed population could require additional safety factors (i.e. FQPA) and thus influence the desired or target MOE.

Science policy issues raised by the FQPA

The implementation of the FQPA raised unique scientific questions that involved a re-assessment of data requirements and the development of novel approaches to pesticide risk assessment. Many of these approaches are currently under development and/or implementation in both the US and Canadian agencies.

Drinking water exposure

All sources of pesticide exposure, including exposure through drinking water, must be factored into decisions on establishing and re-assessing tolerances/maximum residue levels. At present, there are no national databases on pesticide residues in drinking water. The monitoring data that do exist are for a small number of pesticides with a history of intensive use in specific geographical areas. Pesticides are assessed for their ability to contaminate surface and ground water and models developed for predicting the magnitude of pesticide residues in surface and ground water are used for deriving drinking water values. Currently, in the United States the model estimates are compared with a human health drinking water level of concern (DWLOC), which is the theoretical concentration of a pesticide in drinking water that would be an acceptable upper limit in light of the aggregate exposure to that pesticide from other sources (food and residential use). As the models are highly conservative, if the model estimate does not exceed the DWLOC, it can be concluded with reasonable certainty that the contribution from pesticide residues in drinking water does not exceed the Agency's level of concern. More reliable models and comprehensive monitoring programmes are being developed to better refine pesticide exposure in drinking water. In Canada, the PMRA uses a tiered approach for incorporation of drinking water in the dietary assessment. The first tier allocates 10 per cent of the ADI to exposure from drinking water, which is highly conservative. The PMRA also utilizes theoretical concentration estimates derived from models as per the US approach for refining assessments. The PMRA does not pre-set a DWLOC in pre-market assessments. Rather, for products expected to leach into ground water, the PMRA considers mitigative action which may include limitation on use, use rates and geographical restrictions, a requirement for ground water monitoring, or refusal to register.

Aggregate risk assessment

Whereas pesticide exposure from different pathways has been considered previously as independent events, exposure from multiple pathways and relevant routes must now be combined. The routes currently considered in aggregate risk assessments are oral (from food, drinking water, and residential pathways), inhalation (residential pathway), and dermal (residential pathway) (USEPA, 1999). If applicable, the durations of exposure assessed are: acute (one-day exposure), short-term (less than 7

days exposure), intermediate-term (less than 6 months exposure), chronic/long-term (greater than 6 months exposure), and cancer (lifetime exposure). The requirement for an aggregate risk assessment is dependent on several factors, including the pesticide's use pattern, exposure routes, and hazard endpoints. The magnitude of exposure from each pathway, route, and exposure scenario are calculated and then combined to derive a total exposure from the multiple pathways.

Common mechanism of toxicity and cumulative risk assessment

The combined effects (cumulative risk) to human health that can result from pesticides that have a common mechanism of toxicity must now be considered. The cumulative assessment of risk from exposure to multiple chemicals by multiple pathways (food, drinking water, residential exposure to air, soil, outdoor and indoor surfaces) has presented a formidable scientific challenge. Many of the policies for performing such assessments are still being developed (USEPA, 2000b). Prior to considering cumulative effects, aggregate risk assessments are completed for each individual pesticide in a common mechanism group (CMG). If risks for an individual chemical exceed the level of concern, then measures are taken to reduce risks to acceptable levels. A CMG includes chemicals that produce the same toxic effect in the same organ or tissue by the same sequence of major biochemical events. A measure of each chemical's potency relative to other members of the CMG is calculated. At present, the ability to proceed with cumulative risk assessments is limited by inconsistencies in exposure data. While sophisticated probabilistic exposure and risk assessments can be performed for dietary exposure, drinking water and residential exposure estimates are less refined thus posing a challenge for deriving total exposure estimates.

Endocrine disruption

A screening programme to determine whether pesticides and other chemicals may have effects similar to those of naturally occurring hormones has been developed that consists of a two-tiered approach (USEPA, 2000c). Tier 1 screening will identify substances with a potential to interact with the endocrine system of both humans and wildlife. Tier 2 testing will confirm that potential and characterize the effects. Protocols for assays to be used in the tiered testing are presently being validated.

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15 The Regulatory System in Japan

Kannosuke Fujimori

Introduction

Agricultural chemicals (pesticides) as defined in Japan include insecticides, fungicides, growth accelerators, germination depressors, insects used to control plant diseases, and sex pheromones. The use of pesticides brings not only benefit but also concerns about harmful effects on human health and the environment. Three ministries, the Ministry of Agriculture, Forestry and Fisheries (MAFF), the former Ministry of Health and Welfare, now the Ministry of Health, Labour and Welfare (MHLW), and the former Environmental Agency, now the Ministry of the Environment (ME) have responsibilities and obligations in the regulation of safety analysis, risk assessment, and risk management of pesticides in Japan.

Production, marketing, and use of pesticides are regulated under the Agricultural Chemicals Regulation Law enforced by MAFF (MAFF, 1948) Manufacturers and importers cannot sell pesticides unless they are registered. The MHLW is responsible for the protection of public health. This Ministry assesses dietary exposure assessment and is responsible for monitoring pesticide residue levels in crops and drinking water. The main laws under which the MHLW acts are the Food Sanitation Law (MHLW, 1947) and the Drinking Water Law (MHLW, 1957). For each pesticide the MHLW establishes an acceptable daily intake (ADI) based on the toxicological evaluation. Maximum residue limits (MRLs) are established for each crop/pesticide combination from good agricultural practice (GAP) trials. The MHLW establishes that such MRLs are consistent with human health: thus consumption of commodities containing pesticide at the MRL should not cause the ADI to be exceeded. Registration will normally be withheld when the theoretical maximum daily intake for a 50 kg person (TMDI/50 kg) calculated from the MRLs exceeds the ADI.

The ME is responsible for assessing the risk from dietary intake of chemical contaminants from the environment. Water quality in the environment is ensured by standards for water quality or soil in the environment (pesticide residues in soil and water) under the Basic Environment Law (ME, 1993) and the Water Pollution

Prevention Law (ME, 1970). The ME produces standards under which it may cause registration to be withheld on the grounds of potential for environmental contamination. The ME acts under the Agricultural Chemicals Regulation Law as far as MRLs in crops are concerned and also under the Basic Environment Law and Water Supply Law for other purposes.

Safety assessment of pesticides and establishment of the ADI

All the data needed for registration and safety assessment are submitted to the MAFF. The Department of Food Safety, which is part of the Pharmaceutical and Food Safety Bureau of the MHLW, obtains the required data set from the applicant via the MAFF. The data are considered by a meeting of experts: the Expert Meeting for Safety Assessment on Pesticide Residues is convened monthly by the Department of Food Safety. The membership of this expert meeting comprises specialists in the fields of general toxicology, carcinogenicity, genotoxicity, and reproductive toxicology, as well as animal metabolism and food chemistry. Experts from the ME and the MAFF also attend the meeting as observers. The meeting reviews the toxicological data submitted and establishes the ADI for the pesticide from the lowest appropriate no observed adverse effect level (NOAEL) in the most sensitive species using a safety factor. The meeting recommends the ADI to the MHLW.

Toxicological evaluation for the establishment of ADIs in Japan

The Japanese Guidelines for toxicological studies are generally consistent with corresponding OECD guidelines and were revised in 1998 to harmonize with the recently revised OECD guidelines and other test guidelines such as those of the US Environmental Protection Agency (USEPA). Toxicological studies not conducted in compliance with the principle of good laboratory practice are basically not acceptable except for studies on metabolism, mechanistic studies and pharmacological or pharmacodynamic studies, and studies on physiological outcomes. Studies conducted in accordance with guidelines other than Japanese ones, where they differ, such as those of the OECD and USEPA, are acceptable.

NOAELs are ordinarily considered to be critical for safety evaluation in all toxicity studies. Therefore, when a study fails to establish a NOAEL, a newly designed study with appropriate dose ranges may be required. The effects that occur at the lowest observed adverse effect level (LOAEL) are relevant to the evaluation and may determine the safety factor required (see below). The lowest NOAEL in the most sensitive species in the long-term toxicity studies is generally taken as the NOAEL upon which the ADI is based. The ADI is calculated from the

NOAEL, using a safety factor (often of 100), the safety factor taking account of inter- and intra-species differences. Higher safety factors may be required depending on the nature and severity of the toxic effects defining the critical NOAEL.

The studies that are required are acute toxicity studies, short-term toxicity studies, studies of reproduction and teratogenicity, genotoxicity and neurotoxicity, and long-term toxicity and/or carcinogenicity. Additionally, studies of absorption, distribution, excretion, and metabolism are required (see Table 15.1). The list of studies required and their design is almost the same as those required in the OECD guidelines.

Table 15.1 Data requirement on toxicology studies in Japan

Studies	Species	Sample	Data
Acute Oral	≥1	TGAI, FS	Required
Dermal	≥1	TGAI, FS	Required
Inhalation	≥1	TGAI, FS	Required
Primary eye irritation	Rabbit	FS	Required
Dermal irritation	Rabbit	FS	Required
Dermal sensitization	Guinea pig	FS	Required
Acute neurotoxicity	Rat	TGAI	Required
Delayed neurotoxicity	Hen	TGAI	Required[a]
90-day repeat oral toxicity	≥1 Rodent	TGAI	Required
	≥1 Non-rodent	TGAI	Required
21-day repeat dermal toxicity	≥1	TGAI	As necessary
90-day repeat inhalation toxicity	≥1	TGAI	As necessary
Repeat oral neurotoxicity	Rat	TGAI	As necessary
28-day repeat delayed neurotoxicity	Hen	TGAI	As necessary
One-year repeat oral toxicity	≥1 Rodent	TGAI	Required
	≥1 Non-rodent	TGAI	Required
	≥2 Rodent	TGAI	Required
Carcinogenicity toxicity/ carcinogenicity 18-month mouse and 2-year rat	Rat	TGAI	One or the other
Reproductive toxicity	≥1 Rat	TGAI	Required
Teratogenicity	Rat, rabbit	TGAI	Required
Genotoxicity	*in vitro/in vivo*	Not specified	Required
Animal metabolism	Rat or dog	Not specified	Required
General pharmacology	Not specified	Not specified	Required

TGAI = technical grade of active ingredient.
FS = formulated substance.
[a]Expected to inhibit acetylcholinesterase activity.

Establishment of maximum pesticide residue levels (MRLs)

MRLs are estimated using data from field trials conducted according to GAP. MRLs are ordinarily calculated on an individual crop basis, called the Food Standard, and issued by the MHLW. However, when the TMDI exceeds the ADI, the MRL is calculated on crop group basis and called the Standard for Withholding Registration; this is issued by ME. Often MRLs issued by the two Ministries are the same, but the ME may take account of environmental concerns as well as the GAP and hence the MRL may reflect this and differ from that of the MHLW. The MHLW also estimates national TMDIs of pesticides (NTMDIs) in Japan using MRLs and food factors. MRLs for a particular crop are estimated using the data from residue studies conducted under GAP (see above), and the food factor is the mean daily intake of the crop concerned. These data are obtained from national nutritional surveys. Intake of pesticide in drinking water is also included in the calculation of the NTMDI and allocated a maximum of 20 per cent. International standards such as Codex MRLs have been largely accepted by virtue of the SPS agreement (SPS, 1994). However, national MRLs are also used as domestic MRLs for some crops produced in Japan. The reason is that Japanese GAP may be unique in terms of post-harvesting uses, target pests, climate, method of pesticide treatment, and methods of cultivation. Japanese farms are of small size and multiple types of cultivation are often undertaken at a single farm.

The estimated total dietary exposure is in fact thought to be much more than the real pesticide intake level. The MHLW has been calculating national estimated maximum daily intake (N-EMDI) and supervised trials median residue levels (STMRLs) in trials since 1998, in the light of the Codex Committee on Pesticide Residues' recommendations. The N-EMDIs are estimated taking account of the proportion of commodities that are edible, processing and cooking, and other factors where appropriate. The real dietary intake of pesticide residue is calculated based on the data from the National Market Basket Survey. The MHLW calculates the total daily intake of each pesticide, according to the market basket studies. Food groups, including drinking water, are divided to 14 classes. Approximately 100 kinds of foods are collected and cooked by the procedure usually used in Japanese households. The pesticide content in the cooked food is analysed. The intake of the pesticide detected in food is allocated 20 per cent of the detectable limit to the food. The total daily intake is estimated from the amount of the pesticide in the daily intake volume of each food. Six pesticides were detected in foods from the results of market basket studies in 1995 and 1996. At the present time, N-EMDIs are used as reference values for ensuring safety of pesticide residues in food.

The Investigation Council on Pesticide Residues is convened at the request of the Department of Food Safety, and decides whether the recommendation on the ADI

can be adopted, based on a comparison with the TMDI or sometimes the EMDI estimated from MRLs. The council recommends to the Food Sanitation Council that MRLs, which do not cause exceedences of the TMDI or EMDI, should be the Food Standards (MHLW-MRLs [see above]). The Food Sanitation Council submits a report on the Food Standard and/or Standard for Withholding Registration, and other standards involved to the highest council, the Pharmaceutical Affairs and Food Sanitation Council.

Data requirements for registration of pesticides in Japan

Newly produced or newly imported pesticides should be notified to the MAFF for risk assessment of safety in the use of the pesticide and risk assessment of likely residues in food. The primary producer or the importer needs to submit the required data set to the MAFF. The required data set comprises technical specification and chemical identity, proposed pattern of use (direction for use), residue chemistry in crops and the soil, environmental fate in crops, soil, and water, metabolism and toxicological studies, together with a toxicological evaluation. The dossier is reviewed and evaluated for both safety in use and safety of human exposure to residues. All the data described below are not always required.

The specification and chemical identity

The specification will include the chemical and international standard name and code, empirical and structural formulae, physical and chemical properties of the active ingredient, the composition, including the nature of any impurities, the technical grade of the active ingredient, and the nature of co-formulants in the formulation. Information on the stability of the formulated substance under different conditions is also mandatory.

Directions for use

The data on directions for use of the pesticide include use pattern and application procedure, mechanism of action, nature of the target pests, biological efficacy and efficacy on the target pests (insects, weeds, or fungi), and the ecological effects of the pesticide. These last will include information on potentially harmful effects on crops, beneficial insects, fish, birds, and other wild creatures. Ecological data required may include toxicology tests on beneficial insects including LD_{50} or LC_{50} for the honeybee, silkworm and predators/parasites of vermin. Environmental toxicology tests for avian species include LD_{50} for mandarin duck and/or quail (*Coturnix coturnix*). Data on

environmental toxicology tests for aquatic organisms are usually data on LC_{50} for carp, *Daphnia*, other species such as loach (*Cobitidae*), crayfish (*Canbarus*), mullet, rainbow trout (*Salmo gairdnerii*), etc. and data on fish accumulation. Data on environmental toxicology tests for other beneficial animals (such as shellfish and earthworms) or plants (such as algae and surrounding plants) can be required, depending on the situation. In addition to the above, a description of the analytical methods for the pesticide in biological matrices is also required.

Data on residue chemistry (residues in crop and soil)

Residue chemistry data are used to estimate the exposure of the population to the pesticide residue in food and are the results of tests on the amount of residues remaining in food and soil, analysed according to the analytical methods supplied by the applicant for registration. Information on the amount, frequency, and time of pesticide application are necessary to decide the directions for use of the pesticide. Residue data (information on residue in crops and soil) in field and orchard application trials conducted under GAP provided by the MAFF are required. Data on residues in target crops (including orchard crops and crops such as tea, etc.) or residues in crops that are cultivated adjacent to the treated crop are needed. The residue data (including the nature and magnitude of the residue) from at least two supervised trials for each crop using a similar application method to the proposed method of agricultural use are required, together with the analytical method used. Field trials are ordinarily conducted at a minimum of two separate laboratories belonging to national or local government agricultural experimental establishments. Data on residues of pesticide in the soil are required to provide directions for use of the pesticide. Dissipation studies need to be conducted using the samples collected from at least two different types of field (usually from upland and/or paddy fields). Either laboratory soil (in pots) or field soil can be used.

Environmental fate in crops and soil

In Japan, about 60 per cent of agricultural chemicals are used in paddy fields (rice growing in water), while about 40 per cent is used in upland (fields used for growing crops such as wheat, maize, vegetables, or rice, not in water). Data on the environmental fate of the pesticide in crops and soil are required for evaluation of the risk of environmental pollution in food and to non-target species. Information on the fate of the pesticide in plants should include data on absorption, mobility in plants, and major metabolic pathways including photo-reactions and analysis of metabolites. Information on the fate of the pesticide in soils should include data on decomposition, mobility and absorption under aerobic and anaerobic conditions, and, in the case of pesticides susceptible to hydrolysis, the products of hydrolysis and photolysis in water.

Toxicological studies (Table 15.1)

Data requirements in Japan have been revised in accordance with recent changes in the OECD requirements for toxicological, residue, and environmental data. The major revision is the additional requirement for neurotoxicity studies. The toxicological data base which is required for the assessment of the risk in human exposure to pesticide residues in food comprises acute oral toxicity studies, tests on irritation to skin and eye, and tests on skin sensitization. Other tests that are required are oral neurotoxicity studies (acute and repeat study when some evidence is observed in acute or 90-day repeated oral toxicity studies) and 90-day repeat oral toxicity studies. Long-term dietary toxicity studies (one year repeated dose toxicity study and carcinogenicity study or a toxicity/carcinogenicity combined study), reproductive toxicity studies (a two-generation study), and studies of developmental toxicity, a genotoxicity study, an animal metabolic study, and studies of physiological or pharmacological responses to the test chemical. The carcinogenicity study will usually be of at least 18 months duration in mice and 2 years or more in the rat. An oral delayed neurotoxicity study (acute and 28-day repeat when necessary), dermal toxicity studies (acute and 21-day repeat toxicity), and inhalation toxicity studies (90-day repeat toxicity) are required when a class of pesticide or type of exposure give rise to specific concerns about risks to human health.

All the oral toxicity studies and studies of acute exposure, including oral, dermal, and inhalation exposure, must be evaluated on the technical grade active ingredient(s). Formulated products are used in the evaluation of acute toxicity, irritancy, and sensitization. Data on the acute oral toxicity of crop metabolites are required when the intake of the metabolite remaining in an edible portion of the crop gives rise to concerns about risks to human health.

General pharmacology (bio-function) studies

Data on physiological or pharmacological responses to the pesticide are required for registration purposes. The data can provide useful information to predict and characterize possible acute poisonings in humans and to provide information on first aid treatment for intoxication. The studies comprise a detailed clinical observation and tests on pharmacological and toxicological effects of pesticide on major organ systems, such as the central nervous system, the respiratory system, the cardiovascular system, and the renal system. Tests on the functions of other systems, e.g. the autonomic nervous system, skeletal musculature, haematological system, gastrointestinal system may also be required if indicated.

Conclusion

MHLW had established MRLs of 161 pesticides (138 in domestic use) in 130 kinds of crops by 1998. On the other hand, MRLs of 171 pesticides for 12 groups of crop,

78 pesticides for water and soil had been set as Standards for Withholding Registration by the ME before 1998. Thus pesticides are regulated by several standards according to their value in use, degree of exposure and risk assessment for humans, and the environment. The MAFF has registered 309 pesticides, based on the risk assessments of pesticide described above under the Agricultural Chemicals Law (MAFF, 1948). The Standard for Direction of Use is applied to 109 pesticides for crops and 5 pesticides for water. Fifteen pesticides are designated as the objects of standards for quality of drinking water under the Drinking Water Law (MHLW, 1957). Sixteen pesticides are designated as the objects of the standard for water quality in the environment under the Basic Environment Law (ME, 1993). Eight pesticides are designated as objects of the Standards for Soil in Environment under Basic Environment Law. Sixteen pesticides are regulated under the Water Pollution Protection Law (ME, 1970). Standards for Direction of Use are applied to 47 pesticides based on ecological effects, under the Basic Environment Law (ME, 1993). Some of these pesticides are also regulated by other standards or law (such as Poisonous and Deleterious Substance Law), and standards such as those for aerial spray or for pesticides used on golf courses; which standards apply depends on the pattern of use. Pesticides thus are comprehensively regulated in Japan by several Ministries to ensure protection of public health and wildlife, and this is complex because of the variety of Japanese countryside in terms of climate and terrain. Japan has small valleys and plains, short rivers, many high mountains and spreads over many degrees of latitude. Furthermore, Japan is a densely populated country and many highly populated areas are contiguous to areas of food production.

Note: The MHLW has currently established MRLs for 229 pesticides (September 2003).

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Index